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(54) Method for the provision, identification and selection of proteases with altered sensitivity to activity-modulating substances

(57) The present invention provides a method for the selection of proteases with altered sensitivity to one or more activity-modulating substances. The method combines the provision of a protease library encoding polynucleotide sequences, expression of the enzymes, screening of the library in the presence of one or several activity-modulating substances, selection of variants with altered sensitivity to one or several activity-modulating substances and isolation of those polynucleotide sequences that encode for the selected variants.

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Description

[0001] The present invention provides a method for the selection of proteases with altered sensitivity to one or more activity-modulating substances. The method combines the provision of a protease library encoding polynucleotide sequences, expression of the enzymes, screening of the library in the presence of one or several activity-modulating substances, selection of variants with altered sensitivity to one or several activity-modulating substances and isolation of those polynucleotide sequences that encode for the selected variants.

Background of the Invention

[0002] Today many severe medical conditions remain untreatable and require innovative new approaches complementing the traditional medicinal chemistry development of drugs. One alternative emerges from the recent successful introduction of biological therapeutics for the treatment of a number of diseases. Examples for biological therapeutics ("biologics") comprise peptides, proteins, polynucleic acids, lipids or combinations thereof. Traditionally, biologics replaced the bodies own missing or in-active proteins. The potential of biologics has been dramatically broadened by the use of molecules with functions that are not present in the bodies own repertoire, e.g. antibodies directed against a number of targets which are inactivated by binding. In addition, enzymes with different catalytic functions have been developed that increase the rate of a desired reaction with a positive effect on the condition of the patient.

[0003] However, the activity of enzymes is highly regulated in the human or animal body at different levels. For example, the expression of an enzyme may be stimulated by activation of transcription factors, or an enzyme may be activated by a reversible posttranslational modification such as phosphorylation. In signal transduction kinase cascades are known in which upstream kinases phosphorylate and thereby activate downstream kinases. The biological effect is downregulated by the action of phosphatases which remove the phosphate residue and render the kinase inactive. The situation is different for example in proteolytic cascades such as known from the coagulation or complement cascade. The proteases are expressed as inactive proenzymes and are activated by proteolytic cleavage. In this case the downregulation of the protease activity is accomplished by the interaction with inhibitors which are present in blood at high concentrations. The inactivated proteases are degraded and cleared from the bloodstream.

[0004] With respect to applications as biological therapeutics proteases represent a particularly promising example as they can specifically activate or inactivate proteins that are involved in a disease or disease symptoms.

[0005] While antibodies bind targets in a fixed stoichiometry, a protease can activate or inactivate hundreds or thousands of target proteins. Therefore lower doses can be given with the potential of less side effects and lower manufacturing costs. Since nature does not provide proteases which cleave arbitrary targets of interest with sufficient specificity, ways of generating such specific proteases by molecular techniques have been devised. Specificity is an essential element of enzyme function. A cell consists of thousands of different, highly reactive catalysts. Yet the cell is able to maintain a coordinated metabolism and a highly organized three-dimensional structure. This is due in part to the specificity of enzymes, i.e. the selective conversion of their respective substrates. Specificity is a qualitative and a quantitative property. In nature, the specificity of an organism's enzymes has been evolved to the particular needs of the organism. Arbitrary specificities with high value for therapeutic, research, diagnostic, nutritional or industrial applications are unlikely to be found in any organism's enzymatic repertoire due to the large space of possible specificities. Therefore, defined specificities have to be generated *de novo.*

[0006] The application of therapeutic proteases in the treatment of diseases requires their activity in the presence of activity-modulating substances that are present in the application matrix where enzymatic activity is required, e.g. blood serum, extracellular fluid, cerebrospinal fluid, the intracellular environment, or any other environment in the body where activity is required. The serum in particular contains a large variety of protease inhibitors present in high concentrations, most notably serpins (serine protease inhibitors such as alpha1-antitrypsin, antithrombin, antiplasmin, and others) and macroglobulins (such as alpha2-macroglobulin, and others). While serpins inhibit predominantly serine and cysteine proteases, macroglobulins inhibit also other proteases such as metallo proteases.

[0007] There are proteases with lower sensitivity to protease inhibitors then others. A comparatively low sensitivity towards serum inhibitors when comparing it with other human proteases such as trypsin or chymotrypsin has been described for mesotrypsin, a human trypsin variant expressed in the brain and pancreas (Rinderknecht H. et al. Mesotrypsin: A new inhibitor-resistant protease from a zymogen in human pancreatic tissue and fluid. Gastroenterology (1984) 86:681-92). Another example for a protease with comparatively low sensitivity is granzyme B, a serine protease in granules of cytotoxic T-lymphocytes. Kurschus et al. report a 40%-50% residual activity of granzyme B in a solution that corresponds to 80% human serum (Kurschus et al. Killing of target cells by redirected granzyme B in the absence of perforin FEBS Letters (2004) 562:87-92). However, more recent studies have shown that the activity of these proteases in human application matrices containing natural levels of protease inhibitors is not high enough to obtain sufficient activity. And, their specificity is likely to be different from what the application requires.

[0008] Besides the therapeutic use, proteases can be used in industrial, cosmetic, diagnostic or synthetic applications.

To qualify as an effective protease, in particular for therapeutic purposes, proteases should have a low sensitivity, preferably they should be essentially insensitive, to activity-modulating substances present in the targeted application matrices. Therapeutic protease should be insensitive towards different activity-modulating substances to a degree that provides an activity level sufficient to effect its indicated function and at the same time must have sufficient specificity to avoid side effects.

Summary of the Invention

[0009] Thus, the problem underlying the present invention is to provide a method for the selection of proteases with reduced sensitivity towards activity-modulating substances present in the application matrix. This technical problem has been solved by the embodiments of the invention specified below and in the appended claims. The present invention is thus directed to

(1) a method for generating a protease with reduced sensitivity towards activity-modulating substances present within an application matrix, comprising

(a) providing a library of one or more proteases derived from one or more parent proteases,
(b) contacting the proteases with at least one activity-modulating substance, and
(c) selecting one or more protease variants with reduced sensitivity towards activity-modulating substances as compared to the parent protease(s);

(2) the method of (1) above, wherein in step (b) the protease is contacted either simultaneously or consecutively with the activity-modulating substance and at least one substrate, and the turnover of the substrate by the protease under the influence of the activity-modulating substance is determined;
(3) the method of (1) or (2) above, wherein in step (c) the protease variants with reduced sensitivity towards activity-modulating substances are selected by determination of improved IC50 as compared to the parent protease(s);
(4) the method according to (1) to (3) above, wherein the selection in step (c) is achieved by

(i) screening for the residual activity of the protease simultaneous to the contact of the proteases with the activity-modulating substance,
(ii) screening for residual activity of the protease consecutive to the contact of the proteases with the activity-modulating substances, or
(iii) a combination thereof;

(5) the method according to (1) to (4) above, which comprises providing the protease library by surface display expression method, preferably by phage display, and

(i) contacting the displayed proteases with the activity-modulating substances, depleting proteases with sensitivity towards activity modulating substances by immobilisation of complexes comprising the activity-modulating substance and the displayed protease, and selecting for proteases with reduced sensitivity towards activity-modulating substances from the non-immobilized displayed proteases, or
(ii) contacting the displayed proteases with one or more substrates, enriching of proteases with reduced sensitivity towards activity-modulating substances by immobilisation of complexes comprising the substrate and the displayed protease, and selecting for proteases with reduced sensitivity towards activity-modulating substances from the immobilized displayed proteases;

(6) the method according to (1) to (5) above, which further comprises (d) selecting for protease variants in step (c) with the additional selection criteria of substantially similar or altered specificity with regard to the substrate as compared to the parent protease(s), or selecting for protease variants in step (c) and modifying the protease variants obtained in step (c) to exhibit a sustatially similar or altered specificity with regard to the substrate as compared to the parent protease and/or the protease variants obtained in step (c);
(7) the method according to (1) to (6) above, wherein steps (a) to (c) or steps (a) to (d) are repeated cyclically until one or more protease variants with reduced sensitivity towards activity modulating substances are identified, the variants selected in one cycle are used as parent proteases to provide a library for the following cycle, the concentration of the activity-modulating substance is increased from one cycle to the next, and at least two cycles and less than 100 cycles are performed;
(8) the method of (6) above, wherein the modifying step comprises rational design, mutagenesis methods such as random mutagenesis, conversion into a protease scaffold that provides the general catalytic activity with variable

specificity determining regions (SDRs), chemical or genetic fusion to a binding molecule, or any combination thereof;
(9) the method according to (1) to (8) above, wherein in step (b) the proteases are contacted with the activity-modulating substances for 1 s to 72 h, preferably for 30 min to 4 h, before the selection of one or more proteases;
(10) the method according to (1) to (9) above, wherein step (a) comprises providing a library of one or more polynucleotide molecules encoding the proteases, preferably derived form one or more template polynucleotides, by site-specific or random insertion, deletion or substitution of single or multiple mono-nucleotides, polynucleotides, or nucleotide triplets; homologous in vitro recombination; homologous in-vivo recombination; non-homologous recombination; or a combination thereof; and wherein mutagenesis of the polynucleotide templates is performed at or near the active sites of the protease, or at or near the surface of the protease, or random over the entire polynucleotide molecule; or any combination thereof;
(11) the method according to (1) to (10) above, wherein

(i) the library of proteases is expressed in a mammalian or non-mammalian cell-line, viral expression system, yeast, fungi, bacteria, preferably Escherichia coli or Bacillus subtilis, or by use of a cell-free expression system, and wherein genotype and phenotype of the proteases of the population are coupled by use of sample carriers that enable compartmentation of samples, and distributing of genotypes into the sample carriers in a multiplicity per compartment that allows sufficient differentiation of phenotypes; and/or
(ii) the at least one activity-modulating substance contacted with the protease library is provided as isolated component, as part of a fraction of the application matrix or as part of the application matrix, or in any combination thereof; and/or
(iii) the application matrix is derived from a human or animal body fluid selected from the group consisting of blood, digestive fluids, preferably intestinal and gastric juice, mucosa, synovial fluid, interstitial fluid, mucosal fluid, cerebrospinal fluid, peritoneal fluid, or from the extracellular matrix; and/or
(iv) the activity-modulating substance is selected from the table 1, and/or is a human protease inhibitor, preferably a serpin selected from the group consisting of alpha1-antitrypsin, alpha1-antichymotrypsin, kallistatin, protein C-inhibitor, leucocyte elastase inhibitor, plasminogen activator inhibitor, maspin, serpin B6, megsin, serpin B9, serpin B10, serpin B11, serpin B12, serpin B13, antithrombin, heparin cofactor, plasminogen activator inhibitor, alpha-2-plasmin inhibitor, C1-inhibitor, neuroserpin, serpin I2 and thyroxin-binding globulin; a cystein protease inhibitors selected from the group consisting of cystatin A, cystatin B, cystatin C, cystatin D, cystatin E/M, cystatin F, cystatin S, cystatin SA, cystatin SN, cystatin G, kininogen inhibitor unit 2 and kininogen inhibitor unit 3; a metallo protease inhibitor selected from the group consisting of TIMP-1, TIMP-2, TIMP-3 and TIMP-4; macroglobulins, preferably alpha2-macroglobulin; BIRC-1; BIRC-2; BIRC-3; BIRC-4; BIRC-5; BIRC-6; BIRC-7 or BIRC-8;

(12) the method according to (1) to (11) above, wherein the one or more parent proteases

(i) are derived from proteases with a non-naturally occurring catalytic activity of defined specificity engineered by rational design, mutagenesis methods such as random mutagenesis, combination of a protease scaffold that provides the general catalytic activity with variable specificity determining regions (SDRs), chemical or genetic fusion to a binding molecule, or any combination thereof; and/or
(ii) are derived from a protease selected from the group consisting of aspartic, cysteine, serine, metallo and threonine proteases, preferably the protease is derived from a serine protease of the structural class S1, S8, S11, S21, S26, S33 or S51, more preferably from class S1 or S8, a cysteine protease of the structure class C1, C2, C4, C10, C14, C19, C47, C48 or C56, more preferably from class C14, an aspartic protease of the structural class A1, A2 or A26, more preferably from class A1, a metalloprotease of the strucutral class M4 or M10, or the protease is selected from the table 2;

(13) the method of (12) above, wherein the protease is a serine protease of the structural class S1, preferably is a trypsin-like protease, more preferably is derived from a human trypsin, most preferably is derived from the protease according to SEQ ID NO: 5;
(14) the method according to (1) to (13) above, wherein the parent protease or the protease variant selected in step (c) or (d) is fused to

(i) at least one further proteinacious component, preferably said proteinacious component being selected from the group consisting of binding domains, receptors, antibodies, regulation domains, pro-sequences, and fragments thereof, and/or
(ii) at least one further functional component, preferably said further functional component being selected from the group consisting of polyethylenglycols, carbohydrates, lipids, fatty acids, nucleic acids, metals, metal

chelates, and fragments or derivatives thereof;

(15) a protease with reduced sensitivity towards activity-modulating substances obtainable by the method according to (1) to (14) above; (16) the protease of (15) above, which

(i) is derived from a serine protease as defined in (12) or (13) above, preferably is derived from human trypsin having the the amino acid sequence shown in SEQ ID NO:5 or a modified form thereof, and/or
(ii) has one or more mutations at positions that correspond structurally or by amino acid sequence homology to the regions 18-28, 33-41, 48-68 and 129-137 in human trypsin, preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 20-26, 35-29, 50-59, 62-66 and 131-135 in human trypsin, and more preferably at one or more of the following positions 22, 23, 24, 37, 52, 57, 64 and 133 numbered according to the amino acid sequence shown in SEQ ID NO:5; and

(17) the protease of (15) or (16) above, which has at least one mutation selected from Y22T, H23T, F24I or F24V, S37T, E52V, V57A, F64I and D133G, preferably at least 5 mutations selected from S37T, E52V, V57A, F64I and D133G, according to the amino acid sequence shown in SEQ ID NO:5, or mutations at the respective positions which are homologous to said mutations, more preferably has at least three of said mutations, and most preferably is a trypsin mutant of SEQ ID NO:5 having one of the following combination of mutations:

S37T/E52V/V57A/ F64I/D133G,
Y22T/H23T/F24I/S37T/E52V/V57A/F64I/D133G and
Y22T/F24V/S37T/E52V/V57A/F64I/D133G.

Detailed Description of the Drawings

Figure 1 *General scheme of the method for screening and selection of proteases with altered sensitivity to activity-modulating substances*

[0010] A library of polynucleotides coding for a population of proteases is generated from a parent molecule (A). A suitable host is transformed with the polynucleotides (B), cells are dispensed into compartments of a microtiter plate, and the proteins are expressed (C,D). Activity-modulating substances and substrate are added, the activity is measured (E,F,G), and improved variants are selected (H). The improved proteases may represent the basis for a new library of proteases which are subjected to a further round of screening and selection.

Figure 2 *Scheme detailing the basis for selection and iterative improvement of variants*

[0011] The plot shows exemplarily the residual activity of proteases as a function of human blood serum concentration in the protease assay solution. Sigmoidal lines 1 to 9 represent proteases with increasing IC50, i.e. less sensitivity towards the inhibitory effect of human serum. In the example, if the parent protease used to generate the first library has a residual activity at 100% serum that is sufficient to be measured (variant 6; line B), the screen can be performed directly at 100% serum. Improved variants such as number 7 show twice the activity of the parent protease and can be used for the generation of the next library and so forth. Screening and selection is along line C in figure 2. Alternatively, if the residual activity of the parent protease is only measurable at a dilution of 1% (variant 1 and 2; line A) the screen is performed at this low concentration. This first round of screening and selection may yield variant 4 which has measurable activity at 10% serum and therefore screening of the library based on variant 4 can be performed at this higher concentration. In successive rounds the serum concentration n is increased stepwise until variants with the desired properties are obtained.

Figure 3 *Schematic representation of different screening strategies*

[0012] Different embodiments of the screening strategies are depicted schematically. Horizontal bars represent one component of the application matrix and its length is indicative for the concentration. Hatched bars represent activity-modulating substances of the application matrix. I. Screening is performed at concentrations representing 100% of the concentration in the application matrix, for each component. II. All components are present at higher concentration compared to the application matrix and the concentrations are increased in successive rounds. III. All components are present at lower concentration compared to the application matrix and the concentrations are increased in successive rounds IV.

[0013] Individual components of the application matrix are enriched selectively, and the concentration is successively

increased. V. Only one or a few of the components of the application matrix are used in the screen, with successively increasing concentrations. VI. Individual components of the application matrix are depleted compared to the concentration in the application matrix. VII. Each component of the application matrix is present at a certain concentration, be it lower, equal or higher than the corresponding concentration in the application matrix.

Figure 4 *Distributions of activity of a protease library screened at two different serum concentrations*

[0014] Histograms of the activity distribution are shown for a protease library screened at 20% and 50% serum concentration. Under both conditions the activity of the library is clearly distinguished from the negative control. Under less stringent conditions (20% serum) the library distribution shows more activity than under more stringent conditions of (50% serum). Variants with the highest activities are selected for further improvement.

Figure 5 *Determination of serum inhibition in serum for different protease variants*

[0015] Residual activities of different protease variants selected according to the method of the invention were measured in a dilution series of human blood serum. The residual activity was normalized to the uninhibited value and plotted as a function of the serum concentration. The serum concentration at which the activity is 50% corresponds to the IC50 value. The IC50 values increase from variant A to C demonstrating progressive reduction of sensitivity to serum inhibitors.

Figure 6 *Determination of IC50 values with individual inhibitors*

[0016] Residual activity of two protease variants selected according to the method of the invention was measured in a dilution series of alpha2-macroglobulin and antiplasmin, two prominent inhibitors in human blood serum. The maximum concentration of 100% corresponds to the average concentration of the inhibitor in human blood serum, i.e. approximately 1.5 mg/ml for alpha2-macroglobulin and 70 μg/ml for antiplasmin, respectively. The residual activity was normalized to the uninhibited value and plotted as a function of the inhibitor concentration. The inhibitor concentration at which the activity is 50% is the IC50 value. While both variants are relatively insensitive towards alpha2-macroglobulin, sensitivity against antiplasmin is markedly reduced in variant E.

Figure 7 *TNF-alpha inactivation by different protease variants demonstrated in a cell-based assay*

[0017] Different protease variants that were selected according to the method of the invention were incubated with TNF-alpha at increasing serum concentrations. Residual TNF-alpha activity was measured via the capacity to induce apoptosis in a cell-based assay. The control protease trypsin is completely inhibited at the lowest serum concentration. Variant F shows a significantly reduced sensitivity to serum inhibitors, whereas variant G is completely insensitive to serum inhibition.

Detailed Description of the Invention

[0018] In the framework of this invention the following terms and definitions are used.

[0019] The term "**polynucleotide**" corresponds to any genetic material of any length and any sequence, comprising single-stranded and double-stranded DNA and RNA molecules, including regulatory elements, structural genes, groups of genes, plasmids, whole genomes, and fragments thereof.

[0020] The term "**site**" in a polynucleotide or polypeptide refers to a certain position or region in the sequence of the polynucleotide or polypeptide, respectively.

[0021] The term "**position**" in a polynucleotide or polypeptide refers to specific single bases or amino acids in the sequence of the polynucleotide or polypeptide, respectively.

[0022] The term "**region**" in a polynucleotide or polypeptide refers to stretches of several bases or amino acids in the sequence of the polynucleotide or polypeptide, respectively.

[0023] The term "**polypeptide**" comprises proteins such as enzymes, antibodies and the like, medium-length polypeptides such as peptide inhibitors, cytokines and the like, as well as short peptides down to a amino acid sequence length below ten, such as peptidic receptor ligands, peptide hormones, and the like.

[0024] The term "**protease**" means any protein molecule catalyzing the hydrolysis of peptide bonds. It includes naturally-occurring proteolytic enzymes, as well as protease variants. It also comprises any fragment of a proteolytic enzyme, or any molecular complex or fusion protein comprising one of the aforementioned proteins.

The term "**protease variants**" means any protease molecule obtained by site-directed or random mutagenesis, insertion, deletion, recombination and/or any other protein engineering method, that leads to proteases that differ in their amino acid sequence from the parent protease.

[0025] The **"parent protease"** can be either an isolated wild-type protease, or one or more protease variants selected from a library of proteases.

[0026] The term **"protease library"** describes at least one protease variant or a mixture of proteases in which every single protease, resp. every protease variant, is encoded by a different polynucleotide sequence.

[0027] The term **"gene library"** indicates a library of polynucleotides that encodes the library of proteases.

[0028] The term **"isolated"** describes any molecule separated from its natural source.

[0029] The term **"specificity"** means the ability of an enzyme to recognize and convert preferentially certain substrates. Specificity can be expressed qualitatively and quantitatively. "Qualitative specificity" refers to the chemical nature of the substrate residues that are recognized by an enzyme. "Quantitative specificity" refers to the number of substrates that are accepted as substrates. Quantitative specificity can be expressed by the term s, which is defined as the negative logarithm of the number of all accepted substrates divided by the number of all possible substrates. Proteases, for example, that accept preferentially a small portion of all possible peptide substrates have a "high specificity". Proteases that accept almost any peptide substrate have a "low specificity".

[0030] The term **"catalytic activity"** describes quantitatively the conversion of a given substrate under defined reaction conditions.

[0031] The term **"activity-modulating substance"** describes all substances that, when present in the reaction mixture, physically interact with the protease and alter its catalytic activity compared to the activity in the absence of the substance when all other parameters are kept constant. It therefore comprises all modulators, activators and inhibitors of a protease, and all substances that otherwise alter catalytic activity.

[0032] The term **"inhibitor"** describes all substances that, when present in the reaction mixture, physically interact with a protease and decrease its catalytic activity compared to the activity in the absence of the substance when all other parameters and concentrations are kept constant.

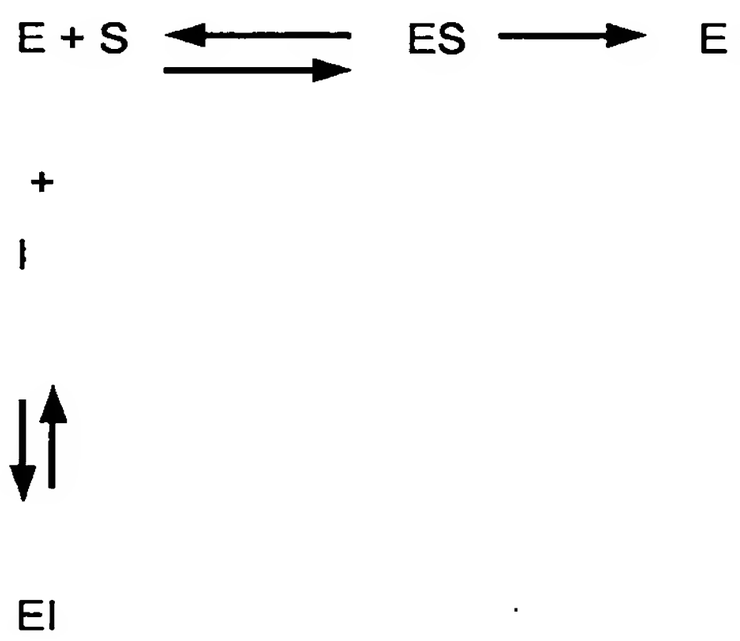
[0033] The term **"activator"** describes all substances that, when present in the reaction mixture, physically interact with a protease and increase its catalytic activity compared to the activity in the absence of the substance when all other parameters and concentrations are kept constant.

[0034] The term **"application matrix"** represents all compositions of molecules, fractions or isolated components that the protease is contacted with at the site where activity is required and during its transfer from the site of first contact with the medium assigned for the specific use and the site where activity of the protease is required. A composition of molecules denotes the entirety of molecules, in particular in their respective combinations and concentrations present at a particular point in space and time. The application matrix comprises both activity-modulating substances, in particular inhibitors or activators, and other activity-modulating substances as well as further components.

[0035] The term **"compartmentation of samples"** describes the coupling of protease genotype and phenotype by use of devices or tools that enable compartmentation of samples. The distribution of genotypes, e.g. into sample carriers is done at a multiplicity per compartment that allows sufficient differentiation of phenotypes.

[0036] The term **"substrate"** or **"peptide substrate"** means any peptide, oligopeptide, or protein molecule of any amino acid composition, sequence or length, that contains a peptide bond that can be hydrolyzed catalytically by a protease. The peptide bond that is hydrolyzed is referred to as the "cleavage site".

[0037] The term **"Ki"** defines the affinity of an inhibitor "I" to the enzyme "E". A general kinetic description for a competitive inhibitor is given by the following scheme, whereby "S" indicates the substrate and "p" the product:



Ki is defined as Ki = [E] [I] / [EI]. It represents the dissociation constant of the inhibitor and the enzyme. A large value

for Ki corresponds to a weak inhibitor, a small value represents a strong inhibitor.

[0038] The term **"residual activity"** is defined defined as the the ratio of the catalytic activity of the enzyme in the presence of an inhibitor (vi) to the catalytic activity in the absence of the inhibitor (v0), all other parameters being equal. Therefore the residual activity a_i is given by $a_i=v_i/v_0$. and a_i*100 is the residual activity in percent. From the above scheme a general equation relating the residual activity to the concentrations of inhibitor and substrate as well as to Km and Ki can be derived:

$$\frac{v_i}{v_0}=\frac{K_m+[S]}{K_m\left(1+\frac{[I]}{K_i}\right)+[S]}$$

where Km = [E] [S] / [ES], Ki = [E] [I] / [EI] and vi/vO *100 represents to the residual acitvity in percent.

[0039] The term **"IC50"** is defined as the concentration of activity-modulating substance at which the activity of a protease is reduced to 50% compared to the activity in the absence of the activity-modulating substance, all other parameters and concentrations being equal. In the context of the equation given above this means that [I]=IC50 when vi/v0=1/2:

$$\frac{1}{2}=\frac{K_m+[S]}{K_m\left(1+\frac{[IC50]}{K_i}\right)+[S]}$$

which can be transformed in the following way

$$2(K_m+[S])=K_m+IC50*\frac{K_m}{K_i}+[S]$$

and

$$K_m+[S]=IC50*\frac{K_m}{K_i}$$

and

$$IC50=(K_m+[S])*\frac{K_i}{K_m}=\left(1+\frac{[S]}{K_m}\right)*K_i$$

[0040] As set forth above the present invention is directed to a method for selection of protease variants with reduced sensitivity towards activity-modulating substances as present in the application matrix of the protease variant. Preferred proteases are aspartic, cysteine, serine, metallo and threonine proteases, even more preferably the protease is a serine protease of the structural class S1, S8, S11, S21, S26, S33 or S51, most preferably from class S1 or S8, a cysteine protease of the structure class C1, C2, C4, C10, C14, C19, C47, C48 or C56, most preferably from class C14, or an

aspartic protease of the structural class A1, A2 or A26, most preferably from class A1, or a metallo protease of the structural class M4 or M10. The protease are also any of the proteases disclosed below in Table 2.

[0041] In a preferred embodiment the protease is derived from human trypsin which is sensitive to a variety of inhibitors in the blood, most notably the serpins. Said proteases may have a desired catalytic activity and or substrate specificity but undesired sensitivity to the activity-modulating substances. The invention provides a method to identify and select proteases with a desired change in the sensitivity against said substances.

[0042] According to the invention this is achieved by providing a protease library derived from one or more parent proteases with desired catalytic activity, contacting said proteases with at least one activity-modulating substance and selecting one or more protease variants with improved IC50 compared to the parent protease(s).

[0043] The first step in selecting proteases with reduced sensitivity towards activity-modulating substances is the generation of libraries of polynucleic acids that encode proteases with different genotypes and/or phenotypes. Different strategies of introducing changes in the coding sequences are applied including but not limited to single or multiple point mutations, exchange of single or multiple nucleotide triplets, insertions or deletions of one or more codons, homologeous or heterologeous recombination between different genes, fusion of additional coding sequences at either end of the encoding sequence or insertion of additional encoding sequences or any combination of these methods. The selection of sites to be mutagenized is based on different strategies as detailed in the following embodiments of the invention. The manipulation of the polynucleic acids to implement these strategies are described in the following embodiments of this first step.

[0044] In a first embodiment the generation of libraries is based on the comparison of two or more genes that are different with respect to the sensitivity towards activity-modulating substances. Changes in the gene of interest are then introduced at sites where the amino acid sequences of the two or more proteases differ. The change can result in substitution of one or more amino acids or randomization at these positions or randomization of amino acids one, two or three amino acids upstream and/or downstream from these positions. The same applies to insertions or deletions of one or more amino acids at such positions or any combination of substitution, insertion and deletion.

[0045] In a further embodiment the strategy is guided by the analysis of the crystal structure, if available, of the complex between the protease and an activity-modulating substance. The distances between atoms belonging to the protease and those belonging to the activity-modulating substance are analyzed and ranked. In a preferred aspect of this embodiment positions are identified that correspond to amino acids whose atoms have a less than a minimal distance to the closest atom of the activity-modulating substance. Either these positions or amino acids in addition to one, two or three amino acids upstream and/or downstream are randomized, or amino acids are inserted or deleted at these positions or any combination of these changes. The minimal distance of the atoms is less than 10 Å. In a more preferred embodiment the minimal distance is less than 5 Å. If no structure of a complex is available such structure is computer modelled from structures of proteases and/or inhibitors that are related to the proteases and/or inhibitors of interest.

[0046] The next embodiment is based on the identification of amino acids which are near the active site and located on the surface of the molecule as preferred sites of mutagenesis. In this embodiment the active site of the protease is identified and a line drawn from the center of mass of the molecule through the center of the active site. A plane perpendicular to this line is approached stepwise from a distant position to the protease towards the open side of the active site. As the plane approaches the protease it will come closer to certain amino acids of the structure. As the plane is approached further it will contact successively more amino acids. The amino acids that are contacted first are the preferred sites for the introduction of mutations. Either these positions or amino acids in addition to one, two or three amino acids upstream and/or downstream are randomized, or amino acids are inserted or deleted at these positions or any combination of these changes.

[0047] In another embodiment the sites targeted for the introduction of changes in the gene are random. Such random point mutations are introduced into the gene of interest by means of mutagenic PCR. Depending on the desired mutation spectrum, this can be accomplished either by a method analogous to the protocol of Cadwell and Joyce (Cadwell RC and Joyce GF. Mutagenic PCR PCR Methods and Applications (1994) 3:136-140; Cadwell RC and Joyce GF. Randomization of Genes by PCR Mutagenesis PCR Methods and Applications (1992) 2:28-33), or by the method of Spee et al. (Spee JH et al. Efficient random mutagenesis method with adjustable mutation frequency by use of PCR and dITP Nucleic Acid Research (1993) 3:777-778), or by similar methods or methods derived thereof.

[0048] According to a further embodiment, primer extension PCR is utilized to introduce certain changes into a gene basically as described by Ho et al. (Ho SN et al. Site-directed mutagenesis by overlap extension using the polymerase chain reaction Gene (1989) 77:51-59 and Horton RM et al. Engineering hybrid genes without the use of restriction proteases: gene splicing by overlap extension Gene (1989) 77:61-68) or a method derived thereof. The method is applied to mutagenize one or more codons, or to insert one or more codons, or to accomplish complete codon mutagenesis.

[0049] In a further embodiment, selective combinatorial randomization (SCR®) is applied for saturating mutagenesis at specific positions within the gene of interest as described in EP 1419248 B1. Using this method, the region to be randomized is determined by a base pair mismatch within a DNA fragment. This can be generated by annealing complementary single strands of different gene variants forming a heteroduplex. The mismatch position is then recognized

and selectively randomized.

[0050] In the next embodiment, several variants which were generated by a method analogous to one or more of the above embodiments are recombined by recombination chain reaction (RCR®) as described in EP 1230390 B1. Using this method, two largely complementary single strands of different gene variants are annealed. The generated heteroduplex is partially digested by an exonuclease and resynthesized with a polymerase, thus adopting the sequence of one strand into the other one during the extension reaction.

[0051] In order to generate enzyme variants with different phenotypes, the libraries of polynucleic acids that encode these different protease variants are translated into proteins by different means.

[0052] Therefore, a suitable host cell is transformed with the encoding polynucleic acid and cultivated under appropriate conditions leading to expression and possible secretion of the protease variant. Different organisms may function as hosts including mammalian or non-mammalian cell lines, microbial organisms or viral expression systems. In a preferred embodiment expression is performed in a microbial system such as yeasts, fungi or bacteria. In a preferred embodiment a bacterial host, preferably *Echerichia coli* or *Bacillus subtilis* is used. Alternatively, the expression is performed applying a viral expression system and in a preferred embodiment a viral display system is used. In addition, a further embodiment comprises in-vitro translation and transcription systems that allow the generation of active protein from the polynucleic acid in the absence of any living organism.

[0053] In another embodiment the coupling between genotype and phenotype is performed by surface display expression methods. Such methods include, for example, phage or viral display, cell surface display and in vitro display. Phage or viral display typically involves fusion of the protease to a viral/phage protein. Cell surface display, i.e. either bacterial or eukaryotic cell display, typically involves fusion of the protease to a peptide or protein that is located at the cell surface. In in-vitro display, the protease is typically made in vitro and linked directly or indirectly to the mRNA encoding the protein (DE 19646372 C1). With phage panning as described by Russel et al. (Russel M, Lowman HB, Clackson T. Introduction to phage biology and phage display, In: Clackson T, Lowman HB, editors. Phage display - a practical approach. Oxford: Oxford University Press; 2004:1-26) the protease is displayed a fusion molecule to a phage surface protein, e.g. as N-terminal part of the gIII surface protein of bacteriophage M13. This can be accomplished by fusing a protease gene library to the C-terminal fragment of the gene gIII and inserting this construct into a phagemid vector. After transformation into an Escherichia coli strain phage particles can be obtained by infection with a helper phage. In a preferred embodiment this procedure is performed with a library of enzyme variants wherein all variants have a defined mutation in the active site rendering the proteases catalytically inactive.

[0054] The recovery of the polynucleic acid that encodes the protease with the desired properties requires a strict coupling of the genotype with the protein and its phenotype.

[0055] In one embodiment this is performed by separating individual transformants of the host cells or individual viruses into isolated compartments of any type followed by cultivation and expression of the protease variants therein. In a preferred embodiment these compartments are given by the individual wells of a micro titer plate, in a more preferred embodiment this is a high-density micro titer plate of any format.

[0056] In another embodiment coupling of genotype and phenotype is obtained by in-vitro transcription and translation of individual polynucleic acids isolated in individual compartments which can be represented by the wells of microtiter plates or droplets of water-in-oil, or water-oil-water emulsions (Tawfik DS and Griffiths AD. Man-made cell-like compartments for molecular evolution Nature Biotechnology (1998) 16:652-656; Bernath K et al. In vitro compartmentalization by double emulsions: sorting and gene enrichment by fluorescence activated cell sorting Analytical Biochemistry (2004) 325:151-157).

[0057] In the second step the multitude of expressed proteases are contacted with the at least one activity-modulating substance. Either simultaneously or consecutively the proteases are contacted with at least one substrate.

[0058] The consecutive contact is preferred when preselection of a subset of proteases that interact to a lower extent or do not interact with the activity-modulating substance is required. Therefore, the proteases are contacted first with the at least one activity-modulating substance and preincubated. Proteases that interact to a lower extent or do not interact with the activity-modulating substance are selected within a subset. This subset of proteases is subsequently contacted with the at least one substrate to identify those variants that are catalytically active. The contact with the at least one substrate is performed either alone or in combination with the activity-modulating substance. In contrast simultaneous contact of the proteases with the at least one activity-modulating substance and the at least one substrate allows the direct determination of the catalytic activity in the presence of the activity-modulating substance.

[0059] The application matrix and therefore the activity-modulating substances depends on the use of the selected proteases. Proteases can be used in industrial, cosmetic, diagnostic or synthetic applications. For these uses the application matrix is given by the composition of any environment relevant for the industrial process, any composition of a cosmetic product or diagnostic reagent, or compositions used in a synthetic application. In one embodiment the proteases are used in the generation of hydrolysates of protein from different plant or animal sources, such as soy, casein and rice. These contain a significant amount of protease inhibitors, e.g. the soy protease inhibitors, Bowman-Birk protease inhibitors (BBI), or soybean trypsin1 inhibitor (SBTI), which reduce the activity of the processing enzyme.

Similarly, proteases are used on bakery to enhance the dough properties. The ingredients of the dough, namely flour, contain inhibitors for proteases and other enzymes. The method of the invention provide proteases with reduced inhibitor sensitivity and favourable process performance.

[0060] A preferred use of the proteases selected by the method of the present invention is as pharmaceutically active substances that reduce or cure the cause or symptoms of a disease. Depending on the indication for which a pharmaceutical protease is intended to be used, catalytic activity is required at different locations in the body. Intended application matrices for pharmaceutical proteases are human or animal body fluids or cytoplasm of cells. The term "body fluid" is not limited to fluids in the strict sense but to all kind of body matrices, such as mucosa, organelles or entire organs. Preferred body fluids include but are not limited to blood, blood serum, blood plasma, digestive fluids such as intestinal and gastric juice and mucosa, synovial fluid, interstitial fluid, mucosal fluid, peritoneal fluid, extracellular matrix, the eye, cerebrospinal fluid, the brain, different organs as well as epithelial and mucosal surfaces of the body and the intracellular space including cytoplasm or cellular organelles such as lysosomes, endosomes, endoplasmic reticulum, Golgi apparatus, nucleus and mitochondria.

[0061] Each of the different application matrices have its particular composition of inhibitors of the enzymatic activity and appropriate proteases with activity in these environments are generated by the method of the invention irrespective of the particular composition of the inhibitors. In a preferred embodiment the proteases are active and insensitive to inhibitors in the blood, synovial fluid or the extracellular matrix.

[0062] The compositions of substances that the proteases are contacted with comprise at least one activity-modulating substance or a mixture of several such substances. Activity-modulating substances can either reduce, enhance or otherwise change the catalytic activity of a protease, e.g. they act as inhibitors or activators of the protease, respectively. In a preferred embodiment of the invention the activity-modulating substances are inhibitors which reduce or eliminate the catalytic activity of the protease.

[0063] The mechanism of inhibition is different for different inhibitors. Some inhibitors are competitive inhibitors, which reversibly bind to the protease. Other inhibitors bind irreversibly to the protease via a covalent bond or the inhibitors are irreversible by practical standards due to an extremely low binding constant. The invention provides a method for the selection of proteases with reduced inhibitor sensitivity independent of the mechanism of inhibition.

[0064] Depending on the intended application matrix, the activity-modulating substances include but are not limited to carbohydrates, lipids, fats, polynucleic acids, peptides and proteins as well as all molecules belonging to the metabolism of the organism in which a therapeutic protease is intended to be used or any combination thereof. In a preferred embodiment the activity-modulating substances are polypeptide or protein inhibitors of the enzymatic function. In a more preferred embodiment the one or more activity-modulating substances are selected from the Table 1 below.

[0065] In a most preferred embodiment the activity-modulating substances are protein inhibitors present in any part of the diseased body for which the protease is intended to be used. These inhibitors include but are not limited to protease inhibitors such as serpins, selected from the group consisting of alpha1-antitrypsin, alpha1-antichymotrypsin, kallistatin, protein C-inhibitor, leucocyte elastase inhibitor, plasminogen activator inhibitor, maspin, serpin B6, megsin, serpin B9, serpin B10, serpin B11, serpin B12, serpin B13, antithrombin, heparin cofactor, plasminogen activator inhibitor, alpha-2-plasmin inhibitor, C1-inhibitor, neuroserpin, serpin I2 and thyroxin-binding globulin; cystein protease inhibitors, selected from the group consisting of cystatin A, cystatin B, cystatin C, cystatin D, cystatin E/M, cystatin F, cystatin S, cystatin SA, cystatin SN, cystatin G, kininogen inhibitor unit 2 and kininogen inhibitor unit 3; metallo protease inhibitors, selected from the group consisting of TIMP-1, TIMP-2, TIMP-3 and TIMP-4; macroglobulins such as alpha2-macroglobulin; BIRC-1, BIRC-2, BIRC-3, BIRC-4, BIRC-5, BIRC-6, BIRC-7 and BIRC-8, and others. Other inhibitors are known to those skilled in the art (Rawling ND et al. Evolutionary families of peptidase inhibitors Biochemistry Journal (2004) 378: 705-716)

[0066] Either simultaneously or consecutively to the contact with the activity-modulating substance the protease variants are contacted with at least one substrate. The substrates include all substances amenable to chemical modification by a protease. These include peptides or proteins as present in the metabolism of an organism. In a preferred embodiment of the invention the substrate is a polypeptide or protein. In a more preferred embodiment the substrate is a protein whose function is relevant for the development of a disease or symptoms. In a most preferred embodiment the protein is a cytokine, such as APRIL, BAFF, BDNF, BMP, CD40-L, EGF, FasL, FGF, Flt3-L, Galectin-3, G-CSF, GM-CSF, IFN-alpha, INF-gamma, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, Leptin, LIGHT, Lymphotactin, M-CSF, MIF, NGF, Oncostatin-M, PDGF, RANKL, RANTES, TGF-alpha, TGF-beta, TNF-alpha, TNF-beta, TRAIL, or VEGF, or their respective receptors.

[0067] In addition the combination of at least one activity-modulating substance and at least one substrate may comprise any further number of substances which are neither activity-modulating nor substrate for the enzymatic activity (non-target molecules). These compositions include but are not limited to all substances of the application matrix of the protease or rather the entire application matrix which already comprises the at least one activity-modulating substance.

[0068] In a third step proteases with reduced sensitivity against the activity-modulating substances are selected. These proteases constitute the parent proteases for the generation of new libraries of protease variants that are subjected

again to the selection process.

[0069] In a first embodiment of this step the concentration of the substances that the variety of proteases are contacted with in the step before is the same as the concentration of substances that are present in the application matrix. This embodiment can be applied when the parent protease has a residual activity that can reliably be measured in the presence of activity-modulating substances at the concentration of the application matrix. For example proteases are contacted with 100% serum, a substrate molecule and more active variants are selected. The complete method of the present invention when iteratively applied leads to variants with a higher activity in the presence of 100% serum than the starting protease.

[0070] In another embodiment the concentration of the activity-modulating substances is equivalent to the concentration of substances in the application matrix and thus the activity may be changed to a level that is outside the dynamic range of the assay format applied. This embodiment provides an approach applicable under these conditions. In such a case the protease variants are contacted with a dilution of the composition of substances in order to reduce the activity-modulating capacity of the composition to an extend that allows the activity of the proteases to be measured within the dynamic range of the assay. In a preferred aspect of this embodiment the dilution leads to concentration of the composition substances in the assay that corresponds to less than 100% of the concentration in the application matrix, more preferred to concentrations of 99%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 1%, 0.1% or less or any concentration in between and most preferred to concentrations from 70% to 5%. Improved variants that are selected at such at reduced concentration represent the basis for the generation of a new library of proteases. These proteases are subsequently contacted with a composition of substances at a concentration higher than the concentration applied to screen the parent proteases. In this iterative process the concentration of the composition of substances including the activity-modulating substances is increased stepwise in each round of the method. This generates proteases with gradually improved properties and allows screening to be performed under conditions where the residual activity of the proteases is within the dynamic range of the assay even in the presence of activity-modulating substances.

[0071] In a further embodiment the concentrations of all substances are increased beyond the concentration present in the application matrix, preferably to 101%, 110%, 120%, 150%, 200%, 300% or more or any concentration in between, more preferably from 120% to 200%. This embodiment provides a means to increase the selective pressure where the activity of proteases is measurable in the presence of 100% of the concentration of substances. In a preferred aspect of this embodiment the concentration of substances is increased stepwise over several cycles beyond the concentration of substances as they occur in the application matrix. In a more preferred aspect the concentrations of serum components in the assay correspond to 101%, 110%, 120%, 150%, 180%, 200%, 250% or 300% or any concentration in between. Most preferably the concentrations range from 120% to 200% of serum.

[0072] In the next embodiment the composition of substances present in the application matrix is selectively depleted of one or more of the activity-modulating substances while the concentrations of all other substances remain unchanged. The extend of depletion is adjusted in order to perform selection of proteases under conditions where the activity-modulating capacity of the composition of substances is reduced to a level that allows the enzymatic activity to fall within the dynamic range of the assay. In following rounds of the iterative optimization process the depletion of said components is reduced stepwise until the full concentration is reached. In a preferred aspect of this embodiment serum is depleted of protease inhibitors by one of several means including but not limited to standard chromatographic procedures such as affinity chromatography to reduce selectively the concentration of protease inhibitors. In one embodiment, molecules with high affinity for the inhibitor, the concentration of which needs to be lowered, are attached to a solid phase. These molecules include but are not limited to antibodies and proteases. In a next step the application matrix is contacted with the immobilized molecule, e.g. either in a batch mode, or a flow column. Alternatively, e.g. serum is incubated with a known amount of a serine protease such as trypsin, chymotrypsin, subtilisin or others which will react with and thereby reduce the concentration of inhibitors such as serpins, in particular alphal-antitrypsin, antithrombin, antiplasmin and others. In successive rounds of optimization the amount of depletion of the activity-modulating substances is decreased stepwise, or it can be replenished at increasing levels.

[0073] In a further embodiment one or several of the components of the application matrix are enriched compared to the concentration of the application matrix. The relative enrichment is increased in successive rounds of screening to provide enhanced selection pressure. The enrichment factor is 101%, 110%, 120%, 150%, 180%, 200%, 250% or 300% of the concentration being present in the application matrix or any concentration in between. Preferred enrichment factors range from 120% to 200% of the concentration.

[0074] In a further embodiment the variety of proteases is contacted with isolated activity-modulating substances or a mixture thereof. The concentration of said activity-modulating substances are lower, equal or higher than the concentration of the respective substances in the application matrix. In a preferred aspect of this embodiment the activity-modulating substances are protease inhibitors. In a more preferred aspect the inhibitors applied are alpha1-anti-trypsin, antithrombin, anti-plasmin or alpha2-macroglobulin or any combination thereof. The concentrations vary from 200% down to 1% of the concentration present in serum.

[0075] All of the embodiments detailed above may optionally include a pre-incubation step of the proteases with the

activity-modulating substances for different lengths of time. In a preferred embodiment of the invention this pre-incubation time is 1 s, 1 min, 10 min, 30 min, 60 min, 2 h, 4 h, 6 h, 12 h, 24 h, 48 h, 72 h or longer, or any time in between. In a more preferred embodiment of the invention the pre-incubation time is 30min, 60min, 2h or 4h.

[0076] In another embodiment the population of proteases is displayed using phage panning. In order to identify proteases with altered sensitivity to activity-modulating substances, the library of phage particles is subject to incubation with one or more activity-modulating substances. Simultaneously or thereafter, the phage suspension is incubated with the substrate. Therefore, the substrate is coupled to a solid phase as is well known in the art for typical phage display targets, e.g. on latex beads, on living cells, in whole organisms or tissues, in multi-well plates, in "immuno-tubes", on a column matrix or a number of other known formats. Such interactions rely on physico-chemical protein-surface interactions. The substrate can also be associated with other substances, but remain in the solution phase. Either type of interaction (solution or solid phase) rely on adsorption or on affinity interactions. There are a number of well known affinity interactions such as binding to specific antibodies, e.g. an anti-target antibody, or specific protein-protein interactions, e.g. antibody-protein A interactions. In a preferred aspect the affinity interactions are mediated by a peptide or chemical "tag" that is added to the target as a peptide fusion such as addition of a (his)6-tag, biotin binding peptide or a FLAG tag, or by posttranslational conjugation such as addition of a biotin tag by chemical conjugation.

[0077] Phage particles presenting a protease which is insensitive to the activity-modulating substance, and possessing specificity for the substrate, bind to the substrate. Where the substrate is in solution (with or without additional associated substances), the substrate is then immobilized by direct capture of the target substrate or indirectly by capture of the associated substance. Where the substrate is bound to a solid phase before panning, this last immobilization step is unnecessary. Once the phage-substrate complex is immobilized, other phages that display more sensitive proteases or display no proteases or display proteases which are less specific, are depleted by washing. Typical washing include washing with solutions of adapted temperature and pH, containing low, medium or high salt, detergent, competitor protein or substrate, competitor phage, and other components. Washings are either done manually or automated, are done continuously or in discrete steps, and can involve changing the washing buffer composition as the washing progresses. The desired phages and thus the desired proteases are thus enriched on the solid phase. The phage is lysed and the encoding DNA recovered, e.g. by cloning or PCR amplification, or the phage is released from the solid phase, e.g., by acid washing, cleavage of the phage away from the protease or other means known in the art and used to infect a host cell for biological amplification. Other specialized selection schemes, such as the use of "selectively infective phage" where the substrate is bound to a ligand needed for host cell infection can also be used in the phage display enrichment of proteases with lowered sensitivity to activity-modulating substances.

[0078] In one aspect of this embodiment, protease variants, each with a defined mutation in the active site rendering the enzyme variants catalytically inactive, are displayed on a phage and selection is performed by panning on immobilised peptid substrates after incubation of the phages with the activity-modulating substances. In a more preferred embodiment such activity-modulating substances are protease inhibitors such as serpins, e.g. alpha1-antitrypsin, antithrombin and antiplasmin, or macroglobulins, e.g. alpha2-macroglobulin.

[0079] In another aspect of this embodiment one or more activity-modulating substances are covalently linked to a solid phase such as latex beads, living cells, whole organisms or tissues, multi-well plates, "immuno-tubes", column matrix or other known solid phases. Then, the phage suspension is incubated with this activity-modulating substance, which preferentially binds phages presenting a protease variant susceptible to the activity-modulating substance and which preferentially leaves the insensitive variants unbound. In a further aspect of this embodiment the activity-modulating substances are left in the solution phase, either alone or associated with other substances in analogy to linkages and associations mentioned above. Then, the activity-modulating substances is immobilized by direct capture of the activity-modulating substances or indirectly by capture of the associated substance. Where the activity-modulating substance was bound to a solid phase before panning, this last immobilization step is unnecessary. The immobilization of the phage-activity-modulating substance complex will preferentially immobilize phage which display proteases that are more sensitive to activity-modulating substances. The non-bound phage, which are enriched for phage displaying proteases that are resistant to activity-modulating substances, are captured by recovering the fluid supernatant. In a preferred aspect this step is repeated once or several times. It is well known in the art that the density of the activity-modulating substances can be a critical parameter. Methods to modify the density or concentration of capture molecules are well known in the art. This "depletion" of undesired phage leaves the supernatant enriched for phage displaying proteases with lowered sensitivity to activity-modulating substances. The two aspects can be also combined, whereby a fraction of the activity-modulating substances is covalently linked and another fraction is left in the solution phase. The ultimately recovered phage are lysed and the encoding DNA recovered, e.g., by cloning or PCR amplification, or the phage are used to infect a host cell for biological amplification.

[0080] Testing of the enzymatic properties is performed in a screening format where variants of the proteases are tested with respect to catalytic activity. In a preferred embodiment the screen is performed in a parallel high-throughput fashion in a miniaturized format in assay volumes less than 1ml. In a more preferred embodiment the volume is less than 100μl, for example 80μl, 60μl, 40μl, 20μl, or 10μl or any volume in between. In an most preferred embodiment

the (well-based) assay volume is less than 10μl, for example 8μl, 6μl, 4μl, 2μl or 1μl or any volume in between. In a further embodiment of the invention the screening volume is less than 1μl, namely 800nl, 600nl, 400nl, 200nl, 100nl or any volume in between.

[0081] In a preferred embodiment the coupling between phenotype and genotype is achieved by distributing individual cells of the transformed host into separated compartments. In a preferred embodiment the compartments are represented by the wells of a micro-titer plate. Variants of the enzymes are expressed in the compartments and contacted with activity-modulating substances and substrate. Activity is measured and variants with improved properties are selected.

[0082] Detection of the enzymatic activity is performed by measuring a physical change accompanied with the modification of the substrate. Changes introduced in the molecule by the modification include changes in activity, size, structure, composition, mass, reactivity, binding characteristics, or chemical properties such as solubility, acidity, color or fluorescence. A change in some of said properties can be measured indirectly by the incorporation of a chemical label into the substrate which changes its properties in response to the enzymatic conversion. In a preferred embodiment one or two fluorescent labels are covalently coupled to the substrate molecule. Substrate conversion is reflected in the change in one or several parameters of the fluorescence such as intensity, anisotropy, fluorescence lifetime, diffusion coefficient, fluorescence energy transfer, fluorescence intensity distribution, fluorescence coincidence analysis or cross-correlation. In a more preferred embodiment the substrate is covalently coupled with a fluorescent label in such a way that proteolytic cleavage leads to a change in the fluorescence anisotropy (EP 1307482). In a most preferred embodiment the proteolytic cleavage of the substrate is monitored by the accompanied loss of biological activity in a cell-based assay. In another preferred embodiment of the invention detection of cleavage of the substrate is performed by separation and detection of proteolytic fragments by chromatography, such as HPLC.

[0083] In another embodiment of the invention the method further comprises the step of selecting for protease variants having substantially similar or higher specificity with regard to the substrate as compared to the parent protease(s). Alternatively the protease variants obtained in step (c) may be modified as to exhibit a catalytic activity of defined specificity, whereby said defined specificity is not or only to a smaller extent occurring in the parent protease and/or the protease variants obtained in step (c). By such additional steps proteases are provided which have a defined specificity for therapeutic, research, diagnostic, nutritional, personal care or industrial purposes. Defined specificity means that the proteases are provided with specificities that do not exist in naturally occurring proteases. The specificities can be chosen by the user so that one or more intended target substrates are preferentially recognized and converted by the proteases. The specificity of proteases, i.e. their ability to recognize and hydrolyze preferentially certain peptide substrates, can be expressed qualitatively and quantitatively. Qualitative specificity refers to the kind of amino acid residues that are accepted by a protease at certain positions of the peptide substrate. For example, trypsin and t-PA are related with respect to their qualitative specificity, since both of them require at the P1 position an arginine or a similar residue. On the other hand, quantitative specificity refers to the relative number of peptide substrates that are accepted as substrates by the protease, or more precisely, to the relative k_{cat}/k_M ratios of the protease for the different peptides that are accepted by the protease. Proteases that accept only a small portion of all possible peptides have a high specificity, whereas the specificity of proteases that, as an extreme, cleave any peptide substrate would theoretically be zero. WO 2004/113521 provides a method for the generation and identification of proteases with desired specificities based on the combination of a protease scaffold that provides the general catalytic activity with variable specificity determining regions (SDRs) which provide the basis for the discrimination between different targets. In addition, the proteases can be fused either on DNA level or chemically to a binding module, e.g. a receptor fragment, an antibody domain or a fragment thereof, to address the target molecule. Furthermore, different mutagenesis methods can be employed to engineer specific proteases, e.g. single or multiple site-directed or random mutagenesis or transfer of amino acid residues or sequence stretches from one protease sequence to another. In another approach the specificity is generated by rational design.

[0084] Before subjected to a further round of screening selected variants may optionally be characterized in more detail with respect to the improved properties. In a preferred embodiment of the invention the IC50 of the variant is determined by incubating it with a serial dilution of the composition of activity-modulating substances and measuring the residual activity.

[0085] Furthermore, the selected protease variants may optionally fused to one or more functional components. These components are preferably proteinacious components which preferably have binding properties to address the target substrate and are of the group consisting of substrate binding domains, antibodies, receptors or fragments thereof. Alternatively, other functional components such as polyethylenglycols, carbohydrates, lipids, fatty acids, nucleic acids, metals, metal chelates, and fragments or derivatives thereof are used.

[0086] The protease variants selected by the method can be used in industrial, cosmetic, diagnostic or synthetic applications. A preferred application of the proteases is the use as therapeutics to reduce or cure the cause or symptoms of a disease. Indications where a protease therapy is beneficial for the patient include inflammation and autoimmune diseases, cancer, cardiovascular diseases, neurodegenerative diseases, allergies, host-versus-graft disease, bacterial or viral infections, metabolic disorders or any other diseases where a protease therapy is indicated. Preferred embodiments of the present invention comprise proteases with beneficial activity for the indications of cancer, inflammation and

autoimmune diseases. A more preferred application is in chronic inflammation.

[0087] Different types of arthritis, rheumatoid arthritis, osteoarthritis, Sjörgen's syndrome, systemic lupus erythematosus, ankylsing spondylitis, psoriasis, inflammatory bowel diseases, Crohn's disease and ulcerative collitis all belong to this area and even today there is a constant need for effective drugs to treat these conditions. In a particularly preferred embodiment the application is rheumatoid arthritis, inflammatory bowel diseases, psoriasis, Crohn's disease, Ulcerative colitis, diabetes type II, classical Hodgkin's Lymphoma (cHL), Grave's disease, Hashimoto's thyroiditis, Sjogren's Syndrome, systemic lupus erythematosus, multiple sclerosis, Systemic inflammatory response syndrome (SIRS) which leads to distant organ damage and multiple organ dysfunction syndrome (MODS), eosinophilia, neurodegenerative disease, stroke, closed head injury, encephalitis, CNS disorders, asthma, rheumatoid arthritis, sepsis, vasodilation, intravascular coagulation and multiple organ failure, as well as other diseases connected with hTNF-alpha.

[0088] Several combinations of the above described embodiments can be defined leading to particular useful variants of the method of the invention. It is understood that the embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art. The invention is further illustrated by the following examples which should not be construed as limiting. The content of all publications, patents, and patent applications cited herein are hereby incorporated by reference.

Experimental Section

Example 1: PCR mutagenesis and library generation

[0089] Random mutagenesis is done by a variation of the standard PCR purification protocol. A PCR reaction is set up in total volume of 100 µl containing a final concentration of 10mM Tris/HCl pH 8.3, 50 mM KCl, 0.01% (wt/vol) gelatin, 7 mM MgCl2, 0.5 mM MnCl2, 0.2 mM dATP and dGTP, 1 mM dCTP and TTP, 0.3 µM of primers P1 and P2, 5 fmol template DNA and 2.5 units Taq polymerase. The following PCR program is used: 95°C 1:00 / 95°C 0:30 / 68°C 0:30 / 72°C 1:00 with 30 PCR cycles from step four to step two.

[0090] Alternatively, mutagenesis at specific sites is done by another variation of the standard PCR protocol. Therefore, two sets of primers are used: primers P1 and P3 binding to 5' and 3' ends of the gene and primers P2 and P4 binding to the site that is to be mutagenited and containing at specific positions mixtures of the four nucleotides. For the first round of PCR, two separate reactions are set up in a total volume of 100 µl, each containing 1 x PCR-Buffer (KOD-Puffer), 0.2 mM dNTPs, 2 mM MgSO4, 0.3 µM of each primer, 20 ng template DNA and 2.5 units KOD polymerase. The following PCR program is used: 94°C 2:00 / 94°C 0:30 /55°C 0:30 / 68°C 0:45 / 6°C for ever with 22 PCR cycles from step four to step two. To generate the first fragment, primers P1 and P3 are employed whereas primers P2 and P4 are used for the second fragment. Consecutively, the two PCR products are separated from remaining template DNA by preparative agarose gel electrophoresis and purified using a gel purification kit (Qiagen). The two PCR products are mixed in an equimolar ratio with a total amount of 100 ng and serves as a template for a extension reaction carried out in a reaction mixture essentially analogous to the one above, with the terminal primers P1 and P2.

Primer:

[0091]

P1: TGGCAGGAGGGGCCACTCAGGCCTTTGCA (SEQ ID NO:1)
P2: CACCTAGTGGCCTAGTCGGCCTTAGC (SEQ ID NO:2)
P3: GATGATCTGCTCATTCCCCTCCAAGGCTCCMNNMNNGTGCACTCCCAGTCTCAC (SEQ ID NO:3)
P4: GGGAATGAGCAGATCATC (SEQ ID N0:4)

[0092] 2 µg of the generated PCR fragment are digested with restriction endonucleases. In a similar approach, 8 µg of a standard plasmid for introducing genes into Bacillus subtilis plasmid (Palva I. et al. Secretion of Eschirichia coli beta-lactamase from Bacillus subtilis by the aid of alpha-amylase signal sequence. Cell Biology (1982) 79:5582-5586) are cut with restrictionendonucleases and dephosphorylated with CIAP. The digest of the plasmid DNA is heated to 50°C followed by phenol-extraction. Finally, the PCR fragment and the plasmid DNA are both purified. Ligation is carried out over night at 16°C according to the manufacturer's instructions (MBI fermentas). After heat-inactivation at 65°C for ten minutes the DNA is subjected to ethanol precipitation, dried and transformed into Escherichia coli cells. The transformed cells are suspended in LB medium and grown over night at 37°C in a shake flask incubator. The plasmid DNA of the generated library is then purified and transformed into Bacillus subtilis according to the protocol of Spizizen (Spizizen J. Transformation of biochemically deficient strains of Bacillus subtilis by deoxyribonucleate. Proc. Natl. Acad. Sci. US (1958) 44:1072-1978) for assessing the individual variants.

Example 2: Screening and selection

[0093] The principle lying behind the selection and screening strategy is illustrated in figure 2. The plot shows the residual activity of variants as a function of serum concentration in the assay solution. Sigmoidal lines 1 to 9 represent variants with increasing IC50, i.e. less sensitivity towards the inhibitory effect of serum. If the parent enzyme used to generate the first library has a residual activity at 100% serum that is sufficient to be measured (variant 6), the screen can be performed directly at full serum concentration. Improved variants such as number 7 show twice the activity of the parent and can be used for the generation of the next library and so forth. Screening and selection is along line C in figure 2. Alternatively, if the residual activity of the parent enzyme is only measurable at a dilution of 1% (variant 1 and 2; line A) the screen is performed at this low concentration. This first round of screening and selection may yield variant 4 which has measurable activity at 10% serum and therefore screening of the library based on variant 4 can be performed at this higher concentration. In successive rounds the serum concentration is increased stepwise until variants with the desired properties are obtained.

[0094] In order to identify enzyme variants having the desired substrate specificity, a screening approach based on a confocal fluorescence spectroscopy set-up as disclosed in WO 9416313 was used. A cell suspension of a Bacillus subtilis in culture medium was dispensed at a cfu-concentration ensuring that single cells are dispensed in each well of the microtiter plate. Cultures are grown over night at 37°C and protein is secreted into the supernatent. Serum is added in a dilution so that the final concentration in the assay allows detection of the enzymatic activity. After adding the substrate (TNF-alpha covalently labelled with a fluorophore) to the sample and incubation for a certain period of time, the samples were subjected to measurement by confocal fluorescence spectroscopy. If necessary, this procedure was repeated several times in order to measure kinetics of the proteolytic cleavage. Samples were ranked according to proteolytic activity, and samples exceeding a certain activity threshold were identified in order to isolate the gene encoding the corresponding protease variant. The distribution of proteolytic activities of protease variants at different concentrations of serum obtained by this procedure is shown in figure 4.

Example 3: Determination of IC50's in human serum

[0095] A more detailed characterization of the inhibitor sensitivity is obtained by the determination of IC50's of different variants. The IC50 is the concentration of inhibitor at which the residual activity is reduced to 50% of the uninhibited value. The enzymes are incubated at a concentration of 1.5 μg/ml in PBS / pH 7.4 /0.1% Triton-X-100 with a serial dilution of human serum in the same buffer, yielding the indicated final concentration. Fluorescently labelled TNF-alpha is added and proteolytic cleavage is followed over time at 37°C by measuring changes in fluorescence parameters. The residual activity is normalized to the uninhibited value and plotted against the serum concentration (figure 5). Clearly, the IC50 values of variants obtained in successive rounds of optimization increase, therefore the variants are less sensitive to the inhibitors present in human serum. The variants A, B and C comprises the following mutations within human trypsin according to the amino acid sequence shown in SEQ ID NO:5:

Variant A: S37T/ E52V/ V57A/ F64I/ D133G
Variant B: Y22T/ H23T/ F24I/ S37T/ E52V/ V57A/ F64I/ D133G
Variant C: Y22T/ F24V/ S37T/ E52V/ V57A/ F64I/ D133G

Example 4: Determination of IC50's of individual inhibitors

[0096] Human serum contains a large number of different protease inhibitors which cannot be differentiated in the experiment described in example 3. To identify inhibitors to which the enzymes are particularly sensitive these can be tested individually. The concentrations of the different inhibitors in the serum vary considerably, between 70 μg/ml for anti-plasmin and 1.5 mg/ml for anti-trypsin and alpha2-macroglobulin. These concentrations are defined as 100% and the enzymes are incubated with dilution series' of the individual inhibitors.

[0097] Fluorescently labelled TNF-alpha is added and proteolytic cleavage is followed over time at 37°C by measuring changes in fluorescence parameters. The residual activity is normalized to the uninhibited value and plotted against the concentration of the inhibitors. Figure 6 shows that the two variants are both relatively insensitive to alpha2-macroglobulin even at the high concentration of about 1.5 mg/ml. However, sensitivity is clearly different towards anti-plasmin. While variant D is 50% inhibited at a concentration that corresponds to about 5% serum, the value for variant E is on the order of 40% serum equivalent. This demonstrates the success of the screening and selection procedure applied.

Example 5: Determination of serum insensitivity in a cell-based assay

[0098] A further demonstration for the decreased sensitivity of successive variants is given in the following example

of a cellular assay. The principle is based on TNF-alpha's capacity to induce apoptosis in susceptible cells. The cascade of events triggered by TNF-alpha binding to the TNF-receptor includes activation of caspase-3. A caspase-3 specific artificial substrate is added which releases aminoluciferin upon cleavage which in turn is the substrate for luciferase. TNF-alpha is incubated with enzyme variants at serum concentrations of 0%, 20%, 40%, 80%, and 100% for 4 h at 37°C. After appropriate dilution (RPMI /10% FCS / 2 mM glutamine / 1 μg/ml actinomycin) the mixture is applied to a culture of WEHI cells and incubated for 6h at 37°C / 5% CO2. Afterwards a ready mix assay reagent from Promega (Caspase-GlowTM-3) is added, 15 min incubated and luminescence detected. Figure 7 shows that trypsin is completely inhibited even by 20% serum while variant F shows a clear decrease in activity with increasing serum concentration. Variant G is unaffected by 100% serum.

Table 1: Activity-modulating substances

Code	Inhibitor name
I01.001	ovomucoid inhibitor unit 1
I01.002	ovomucoid inhibitor unit 2
I01.003	ovomucoid inhibitor unit 3
I01.004	ovoinhibitor inhibitor unit 1
I01.005	ovoinhibitor inhibitor unit 2
I01.006	ovoinhibitor inhibitor unit 3
I01.007	ovoinhibitor inhibitor unit 4
I01.008	ovoinhibitor inhibitor unit 5
I01.009	ovoinhibitor inhibitor unit 6
I01.010	ovoinhibitor inhibitor unit 7
I01.011	SPINK1
I01.012	SPINK2
I01.013	SPINK5 inhibitor unit 1
I01.014	BUSI-I inhibitor
I01.015	BUSI-II inhibitor
I01.016	bikazin salivary inhibitor inhibitor unit 1
I01.017	bikazin salivary inhibitor inhibitor unit 2
I01.018	elastase inhibitor (Anemonia sulcata)
I01.019	rhodniin inhibitor unit 1
I01.020	bdellin
I01.021	tryptase inhibitor (Hirudo medicinalis)
I01.022	dipetalogastin inhibitor unit 2
I01.023	dipetalogastin inhibitor unit 3
I01.024	TgPI inhibitor inhibitor unit 1 (Toxoplasma gondii)
I01.025	TgPI inhibitor inhibitor unit 2 (Toxoplasma gondii)
I01.026	TgPI inhibitor inhibitor unit 3 (Toxoplasma gondii)
I01.027	TgPI inhibitor inhibitor unit 4 (Toxoplasma gondii)
I01.028	SPINK5 inhibitor unit 2
I01.029	SPINK5 inhibitor unit 3
I01.030	SPINK5 inhibitor unit 4
I01.031	rhodniin inhibitor unit 2

(continued)

Code	Inhibitor name
I01.032	SPINK5 inhibitor unit 6
I01.033	NcPI-S protein (Neospora caninum)
I01.034	EPI1 protein inhibitor domain (Phytophthora infestans)
I01.035	skin protein 1 (Phyllomedusa sauvagii)
I01.036	dipetalogastin inhibitor unit 1
I01.037	RECK protein inhibitor unit 1
I01.038	infestin 4
I01.039	PAPI I inhibitor unit (Pacifastacus leniusculus)
I02.001	aprotinin
I02.002	spleen trypsin inhibitor I (Bos taurus)
I02.003	colostrum trypsin inhibitor (Bos taurus)
I02.004	serum basic peptidase inhibitor (Bos taurus)
I02.005	bikunin inhibitor unit 1
I02.006	bikunin inhibitor unit 2
I02.007	hepatocyte growth factor activator inhibitor 1 inhibitor unit 1
I02.008	hepatocyte growth factor activator inhibitor 1 inhibitor unit 2
I02.009	hepatocyte growth factor activator inhibitor 2 inhibitor unit 1
I02.010	hepatocyte growth factor activator inhibitor 2 inhibitor unit 2
I02.011	tissue factor pathway inhibitor-1 inhibitor unit K1
I02.012	tissue factor pathway inhibitor-1 inhibitor unit K2
I02.013	tissue factor pathway inhibitor-2 inhibitor unit K1
I02.014	tissue factor pathway inhibitor-2 inhibitor unit K2
I02.015	protease nexin II
I02.016	amyloid-like protein 2
I02.017	peptidase inhibitor (Tachypleus)
I02.018	chymotrypsin inhibitor SCI-I (Bombyx mori)
I02.019	paragonial peptide D (Drosophila funebris)
I02.020	boophilin inhibitor unit 1
I02.021	boophilin inhibitor unit 2
I02.022	chelonianin inhibitor unit 1
I02.023	carrapatin
I02.024	ornithodorin inhibitor unit 1
I02.025	ixolaris inhibitor unit
I02.026	peptidase inhibitor 5 (Anemonia sulcata)
I02.032	ornithodorin inhibitor unit 2
I02.033	WFIKKN peptidase inhibitor inhibitor unit 2
I02.034	Ac-KPI-1 I (Ancylostoma caninum) inhibitor unit
I02.035	savignin (Ornithodoros savignyi)

(continued)

Code	Inhibitor name
I02.037	Kil-1 g.p. (Drosophila virilis)
I02.039	amblin inhibitor unit (Amblyomma hebraeum)
I03.001	soybean trypsin inhibitor
I03.002	cathepsin D inhibitor (Solanum tuberosum)
I03.003	trypsin/chymotrypsin inhibitor (Alocasia macrorrhiza)
I03.004	alpha-amylase/subtilisin inhibitor (Hordeum vulgare)
I03.005	chymotrypsin inhibitor ECI (Erythrina variegata)
I03.006	proteinase inhibitor A (Sagittaria sagittifolia) inhibitor unit
I03.007	proteinase inhibitor B (Sagittaria sagittifolia) inhibitor unit
I03.008	trypsin inhibitor (Enterolobium contortisiliquum)
I03.009	winged-bean chymotrypsin inhibitor
I03.010	trypsin inhibitor (Acacia confusa)
I03.011	erythrina trypsin/tissue plasminogen activator inhibitor
I03.012	cruzipain inhibitor (Bauhinia bauhinioides)
I03.013	sporamin
I03.015	AtDr4 g.p. (Arabidopsis thaliana)
I03.016	bauhinia trypsin/plasma kallikrein inhibitor
I03.017	cysteine protease inhibitor 1 (potato)
I03.018	trypsin inhibitor PtTI (Populus tremuloides)
I03.019	papain inhibitor (Prosopis juliflora)
I03.020	potato Kunitz-type trypsin inhibitor
I03.021	Kunitz serine peptidase inhibitor (Delonix regia)
I03.022	latex serine peptidase inhibitor (Papaya carica)
I04.001	alpha-1-peptidase inhibitor
I04.002	alpha-1-antichymotrypsin
I04.003	kallistatin
I04.004	protein C inhibitor
I04.005	protein Z-dependent peptidase inhibitor
I04.006	serpin B1
I04.007	plasminogen activator inhibitor 2
I04.008	squamous cell carcinoma antigen 1
I04.009	squamous cell carcinoma antigen 2
I04.010	maspin
I04.011	serpin B6
I04.012	megsin
I04.013	serpin B8
I04.014	serpin B9
I04.015	serpin B10

(continued)

Code	Inhibitor name
I04.016	serpin B12
I04.017	serpin B13
I04.018	antithrombin
I04.019	heparin cofactor II
I04.020	plasminogen activator inhibitor-1
I04.021	protease nexin I
I04.022	pigment epithelium derived factor
I04.023	alpha-2-plasmin inhibitor
I04.024	C1 inhibitor
I04.025	neuroserpin
I04.026	serpin I2
I04.027	endopin 1 (Bos taurus)
I04.028	viral serpin
I04.029	contrapsin
I04.030	peptidase inhibitor 3 (rodent)
I04.031	alaserpin (Lepidoptera)
I04.032	barley-type serpin
I04.033	myeloid and erythroid nuclear termination stage-specific protein (Gallus gallus)
I04.034	endopin 2 (Bos taurus)
I04.035	colligin 1
I04.036	colligin 2
I04.037	thermopin (Thermobifida fusca)
I04.038	PAP-regulating serpin (insect)
I04.039	serpin SP6 (Drosophila melanogaster)
I04.040	serpin Spn43Ac (Drosophila melanogaster)
I04.041	serpin SRP-2 (Caenorhabditis elegans)
I04.042	serpinb3b (Mus musculus)
I04.043	serpin 4 (Drosophila melanogaster)
I04.044	serpin SPI-C1 (Mus musculus)
I04.045	serpin SPI2 (Mus musculus)
I04.952	Homologue: serpin A2
I04.953	Homologue: angiotensinogen
I04.954	Homologue: corticosteroid-binding globulin
I04.955	Homologue: thyroxin-binding globulin
I04.955	Homologue: Homologue: thyroxin-binding globulin
I04.955	Homologue: Homologue: Homologue: thyroxin-binding globulin
I04.955	Homologue: Homologue: Homologue: Homologue: thyroxin-binding globulin
I04.956	Homologue: serpin B11

(continued)

Code	Inhibitor name
I04.958	Homologue: ovalbumin
I05.001	ascidian trypsin inhibitor
I06.001	maize trypsin/factor XIIa inhibitor
I06.002	barley trypsin/factor XIIa inhibitor
I06.003	ragi seed trypsin/alpha-amylase inhibitor
I06.004	wheat trypsin/alpha-amylase inhibitor
I07.001	trypsin inhibitor MCTI-1 (Momordica charantia)
I07.002	elastase inhibitor MCEI (Momordica charantia)
I07.003	trypsin inhibitor EETI-II (Ecballium elaterium)
I07.004	macrocyclic trypsin inhibitor (Momordica cochinchinensis)
I07.005	trypsin inhibitor CMTI-I (Cucurbita maxima)
I07.006	trypsin inhibitor CPTI (Cucurbita pepo)
I07.007	trypsin inhibitor CMTI-III (Cucurbita maxima)
I07.008	trypsin inhibitor MCTI-II (Momordica charantia)
I07.009	trypsin inhibitor CVTI-I (Cucurbitaceae)
I07.010	trypsin inhibitor TGTI-I (Luffa cylindrica)
I07.011	trypsin inhibitor TGTI-II (Luffa cylindrica)
I07.012	trypsin inhibitor LCTI (Luffa cylindrica)
I07.013	trypsin inhibitor CMTI-II (Cucumis melo)
I07.014	trypsin inhibitor CSTI-IIb (Cucumis sativus)
I07.015	trypsin inhibitor CSTI-IV (Cucumis sativus)
I07.016	trypsin inhibitor BDTI-II (Bryonia dioica)
I07.017	trypsin inhibitor MRTI-I (Momordica repens)
I07.018	trypsin inhibitor MCTI-A (Momordica charantia)
I07.019	trypsin inhibitor CMeTI-B (Cucumis melo)
I07.020	trypsin inhibitor TTII (Trichosanthes kirilowii)
I07.021	trypsin inhibitor MCTI-III (Momordica charantia)
I08.001	chymotrypsin/elastase inhibitor (Ascaris-type)
I08.002	Acp62F protein (Drosophila melanogaster)
I08.003	Bombina trypsin inhibitor
I08.004	hookworm coagulation inhibitor
I08.005	coagulation inhibitor (Anisakis simplex)
I08.006	inducible metallopeptidase inhibitor (Galleria mellonella)
I08.007	Ascaris trypsin inhibitor
I08.008	cathepsin G/chymotrypsin inhibitor (Apis mellifera)
I08.950	Homologue: von Willebrand factor
I08.951	Homologue: mucin
I08.952	Homologue: mucin 6

(continued)

Code	Inhibitor name
I08.953	Homologue: mucin 5B
I09.001	subtilisin propeptide
I09.002	peptidase A inhibitor 1 (Pleurotus ostreatus)
I09.003	endopeptidase B inhibitor (fungus)
I10.001	marinostatin
I11.001	ecotin
I12.001	lima bean-type trypsin inhibitor
I12.002	sunflower cyclic trypsin inhibitor
I12.003	Bowman-Birk trypsin inhibitor (Medicago-type)
I12.004	Bowman-Birk elastase and trypsin inhibitor (Phaseolus-type)
I12.005	Bowman-Birk inhibitor (Glycine-type) unit 1
I12.006	Bowman-Birk trypsin/chymotrypsin inhibitor (Arachis hypogaea)
I12.007	rice Bowman-Birk inhibitor inhibitor unit 1
I12.008	Bowman-Birk inhibitor (Glycine-type) unit 2
I12.009	Bowman-Birk inhibitor (Gramineae) inhibitor unit 1
I12.010	Bowman-Birk inhibitor (Gramineae) inhibitor unit 2
I12.011	rice Bowman-Birk trypsin inhibitor inhibitor unit 2
I12.012	rice Bowman-Birk trypsin inhibitor inhibitor unit 3
I12.013	rice Bowman-Birk trypsin inhibitor inhibitor unit 4
I12.014	bromelain inhibitor (Ananas comosus)
I12.015	wheat-germ trypsin inhibitor (Triticum aestivum) inhibitor unit 1
I12.016	wheat-germ trypsin inhibitor (Triticum aestivum) inhibitor unit 2
I13.001	eglin C
I13.002	potato peptidase inhibitor I
I13.003	chymotrypsin inhibitor 2
I13.004	glutamyl endopeptidase II inhibitor (bitter gourd)
I13.005	subtilisin-chymotrypsin inhibitor CI-1A (barley)
I13.006	chymotrypsin inhibitor I (potato)
I13.007	subtilisin inhibitor I (Fabaceae)
I13.008	inhibitor of trypsin and Hageman factor (Cucurbita maxima)
I13.009	trypsin/subtilisin inhibitor (Amaranthus sp.)
I13.010	tomato peptidase inhibitor I
I13.011	buckwheat peptidase inhibitor I
I13.012	wheat subtilisin/chymotrypsin inhibitor
I13.013	cytin B chain (Theromyzon tessulatum)
I14.001	hirudin
I14.002	haemadin
I15.001	hirustasin

(continued)

Code	Inhibitor name
I15.002	bdellastasin
I15.003	theromin (Theromyzon tessulatum)
I15. 004	tessulin
I15.005	guamerin (Hirudo nipponia)
I15.006	therin
I15.007	antistasin inhibitor unit 1
I15.008	antistasin inhibitor unit 2
I15.009	ghilanten inhibitor unit 1
I15.010	ghilanten inhibitor unit 2
I15.011	cytin A chain (Theromyzon tessulatum)
I16.001	plasminostreptin
I16.002	kexstatin I
I16.003	streptomyces subtilisin inhibitor
I16.004	SIL1 inhibitor (Streptomyces cacaoi)
I16.005	SIL8 inhibitor
I16.006	trypsin inhibitor STI1 (Streptomyces sp.)
I17.001	mucus peptidase inhibitor inhibitor unit 2
I17.002	elafin
I17.003	huWAP2
I17.004	chelonianin inhibitor unit 2
I17.950	Homologue: mucus peptidase inhibitor inhibitor unit 1
I18.001	mustard trypsin inhibitor
I18.002	rape trypsin inhibitor
I19.001	peptidase inhibitor LMPI (Orthoptera) inhibitor unit 1
I19.002	pacifastin inhibitor unit 1
I19.003	pacifastin inhibitor unit 2
I19.004	pacifastin inhibitor unit 3
I19.005	pacifastin inhibitor unit 4
I19.006	pacifastin inhibitor unit 5
I19.007	pacifastin inhibitor unit 6
I19.008	pacifastin inhibitor unit 7
I19.009	pacifastin inhibitor unit 8
I19.010	pacifastin inhibitor unit 9
I19.011	peptidase inhibitor LGPI (Orthoptera) inhibitor unit 2
I20.001	potato peptidase inhibitor II inhibitor unit
I20.002	tobacco peptidase inhibitor II inhibitor unit
I20.003	tomato peptidase inhibitor II inhibitor unit
I20.004	serine peptidase inhibitor II (Capsicum-type)

(continued)

Code	Inhibitor name
I21.001	secretogranin V
I24.001	pinA Lon peptidase inhibitor (phage T4)
I25.001	cystatin A
I25.003	cystatin B
I25.004	cystatin C
I25.005	cystatin D
I25.006	cystatin E/M
I25.007	cystatin F
I25.008	cystatin S
I25.009	cystatin SA
I25.010	cystatin SN
I25.011	ovocystatin
I25.012	snake venom cystatin (Bitis sp.)
I25.013	sarcocystatin
I25.014	phytocystatin
I25.015	potato multicystatin inhibitor unit
I25.016	kininogen inhibitor unit 2
I25.017	kininogen inhibitor unit 3
I25.018	T-kininogen inhibitor unit 2
I25.019	T-kininogen inhibitor unit 3
I25.020	alpha-2-HS-glycoprotein inhibitor unit 1
I25.021	alpha-2-HS-glycoprotein inhibitor unit 2
I25.022	histidine-rich glycoprotein inhibitor unit 1
I25.023	cystatin SC
I25.024	cystatin TE-1
I25.025	histidine-rich glycoprotein inhibitor unit 2
I25.026	metallopeptidase inhibitor (snake venom)
I25.027	cystatin G
I25.028	oryzacystatin II
I25.029	sunflower multicystatin inhibitor unit
I25.030	papaya cystatin
I25.031	onchocystatin
I25.950	Homologue: kininogen inhibitor unit 1
I27.001	calpastatin inhibitor unit 1
I27.002	calpastatin inhibitor unit 2
I27.003	calpastatin inhibitor unit 3
I27.004	calpastatin inhibitor unit 4
I29.001	cathepsin L propeptide

(continued)

Code	Inhibitor name
I29.002	cytotoxic T-lymphocyte antigen-2 alpha
I29.003	cathepsin H propeptide
I29.004	cathepsin S propeptide
I29.005	Bombyx cysteine peptidase inhibitor
I29.006	salarin inhibitor unit
I29.007	cathepsin K propeptide
I29.008	cytotoxic T-lymphocyte antigen-2 beta
I29.009	cer g.p. (Drosophila melanogaster)
I31.001	chum salmon egg cysteine peptidase inhibitor
I31.002	MHC II invariant chain p41 form
I31.003	equistatin (Actinia) inhibitor unit 1
I31.004	equistatin (Actinia) inhibitor unit 2
I31.005	equistatin (Actinia) inhibitor unit 3
I31.006	testican-1
I31.950	Homologue: thyroglobulin
I31.951	Homologue: insulin-like growth factor binding protein
I31.952	Homologue: insulin-like growth factor binding protein 3
I31.953	Homologue: insulin-like growth factor binding protein 2
I32.001	BIRC-1 protein
I32.002	BIRC-2 protein
I32.003	BIRC-3 protein
I32.004	X-linked inhibitor of apoptosis protein
I32.005	BIRC-5 protein
I32.006	BIRC-6 protein
I32.007	BIRC-7 protein
I32.008	BIRC-8 protein
I32.009	DIAPI (Drosophila melanogaster)
I33.001	aspin
I34.001	saccharopepsin inhibitor
I35.001	timp-1
I35.002	timp-2
I35.003	timp-3
I35.004	timp-4
I35.005	timp-DM (Drosophila melanogaster)
I36.001	Streptomyces metallopeptidase inhibitor
I37.001	potato carboxypeptidase inhibitor
138.001	metallopeptidase inhibitor Erwinia
138.002	aprin

(continued)

Code	Inhibitor name
138.003	serralysin inhibitor (Serratia sp.)
139.001	alpha-2-macroglobulin
139.002	ovomacroglobulin
139.003	pregnancy-zone protein
139.004	murinoglobulin 1
139.005	murinoglobulin 2
139.006	antigen CD109
139.950	Homologue: complement component C3
139.951	Homologue: complement component C4
139.952	Homologue: complement component C5
I40.001	Bombyx subtilisin inhibitor
I42.001	chagasin
I43.001	oprin
I44.001	carboxypeptidase A inhibitor (Ascaris suum)
I46.001	leech carboxypeptidase inhibitor
I47.001	latexin
I48.001	clitocypin
I49.001	proSAAS
I50.001	baculovirus p35 caspase inhibitor
I50.002	baculovirus p49 caspase inhibitor
I51.001	carboxypeptidase Y inhibitor (Saccharomyces cerevisiae)
I51.002	phosphatidylethanolamine-binding protein
I52.001	tick anticoagulant peptide (Ornithodorus sp.)
I57.001	staphostatin B
I58.001	staphostatin A
I59.001	triabin
I63.001	pro-eosinophil major basic protein
I64.001	thrombostasin (Haematobia irritans)
LI01-001	ovomucoid
LI01-002	ovoinhibitor
LI01-003	bikazin
LI01-004	SPINK5 g.p. (Homo sapiens)
LI01-005	inhibitor TgPI (Toxoplasma gondii)
LI01-006	dipetalogastin
LI01-007	rhodniin
LI01-008	infestin (Triatoma infestans)
LI01-009	PAPI I inhibitor (Pacifastacus leniusculus)
LI02-001	bikunin

(continued)

Code	Inhibitor name
LI02-002	tissue factor pathway inhibitor 1
LI02-003	tissue factor pathway inhibitor 2
LI02-004	hepatocyte growth factor activator inhibitor type 1
LI02-005	hepatocyte growth factor activator inhibitor type 2
LI02-006	boophilin
LI02-007	ixolaris (Ixodes scapularis)
LI02-010	amblin (Amblyomma hebraeum)
LI03-001	proteinase inhibitor B (Sagittaria sagittifolia)
LI12-001	compound inhibitor: I12.005, I12.008
LI12-002	compound inhibitor: I12.009, I12.010, I12.010, I12 unassigned
LI12-004	rice Bowman-Birk inhibitor
LI12-005	compound inhibitor: I12.015, I12.016
LI12-UPW	unassigned compound peptidase inhibitor containing family I12 units
LI15-002	antistasin
LI15-003	ghilanten
LI17-001	mucus peptidase inhibitor
LI19-001	pacifastin
LI20-002	tobacco type 2 peptidase inhibitor
LI20-003	tomato type 2 peptidase inhibitor
LI25-001	multicystatin (potato)
LI25-002	L-kininogen
LI25-003	T-kininogen
LI25-005	histidine-rich glycoprotein
LI25-006	sunflower multicystatin
LI27-001	calpastatin
LI31-001	equistatin
LI90-001	WFIKKN peptidase inhibitor
LI90-002	WFIKKNRP putative peptidase inhibitor
LI90-004	eppin

Table 2: Proteasen

CODE	Protease name
A01.001	pepsin A
A01.002	pepsin B
A01.003	gastricsin
A01.004	memapsin-2
A01.006	chymosin
A01.007	renin

(continued)

CODE	Protease name
A01.008	renin-2
A01.009	cathepsin D
A01.010	cathepsin E
A01.011	penicillopepsin
A01.012	rhizopuspepsin
A01.013	mucorpepsin
A01.014	candidapepsin SAP1
A01.015	barrierpepsin
A01.016	aspergillopepsin I
A01.017	endothiapepsin
A01.018	saccharopepsin
A01.019	polyporopepsin
A01.020	phytepsin
A01.021	plasmepsin (Plasmodium sp.)
A01.022	plasmepsin 1
A01.023	plasmepsin 2
A01.025	peptidase E
A01.026	peptidase F
A01.027	trichodermapepsin
A01.028	embryonic pepsin (Gallus gallus)
A01.029	neurosporapepsin
A01.030	yapsin 1
A01.031	yapsin 2
A01.035	yapsin 3
A01.036	acid peptidase (Yarrowia lipolytica)
A01.037	canditropsin
A01.038	candiparapsin
A01.040	nepenthesin
A01.041	memapsin-1
A01.042	syncephapepsin
A01.043	histoaspartic peptidase (Plasmodium falciparum)
A01.044	podosporapepsin
A01.045	nothepsin
A01.046	napsin A (human-type)
A01.049	napsin A (mouse-type)
A01.050	CND41 peptidase
A01.051	pepsin F
A01.053	nemepsin-3

(continued)

CODE	Protease name
A01.056	Yps1 protein (Schizosaccharomyces pombe)
A01.058	eimepsin
A01.059	plasmepsin 4
A01.060	candidapepsin SAP2
A01.061	candidapepsin SAP3
A01.062	candidapepsin SAP4
A01.063	candidapepsin SAPS
A01.064	candidapepsin SAP6
A01.065	candidapepsin SAP7
A01.066	candidapepsin SAP8
A01.067	candidapepsin SAP9
A01.068	nemepsin-2
A01.069	CDR1 g.p. (Arabidopsis thaliana)
A01.070	pepsin A4 (Homo sapiens)
A01.071	pepsin A5 (Homo sapiens)
A01.072	oryzepsin
A01.073	nucellin
A01.074	AtASP38 peptidase (Arabidopsis thaliana)
A02.001	HIV-1 retropepsin
A02.002	HIV-2 retropepsin
A02.003	simian immunodeficiency virus retropepsin
A02.004	equine infectious anaemia virus retropepsin
A02.005	bovine immunodeficiency virus retropepsin
A02.006	Visna lentivirus-type retropepsin
A02.007	feline immunodeficiency virus retropepsin
A02.008	Moloney murine leukemia virus-type retropepsin
A02.009	Mason-Pfizer leukemia virus retropepsin
A02.010	mouse mammary tumor virus retropepsin
A02.011	human endogenous retrovirus K retropepsin
A02.012	retropepsin (human T-cell leukemia virus)
A02.013	bovine leukemia virus retropepsin
A02.015	Rous sarcoma virus retropepsin
A02.016	intracisternal A-particle retropepsin
A02.018	simian T-cell lymphotropic virus retropepsin
A02.019	multiple-sclerosis-associated retrovirus retropepsin
A02.020	porcine endogenous retrovirus endopeptidase
A02.021	Gypsy transposon (Drosophila sp.) endopeptidase
A02.022	Ty3 transposon (Saccharomyces cerevisiae) endopeptidase

(continued)

CODE	Protease name
A02.024	rabbit endogenous retrovirus endopeptidase
A02.051	retrotransposon peptidase (fungus)
A02.052	retrotransposon 17.6 peptidase
A02.053	S71-related human endogenous retropepsin
A02.054	Osvaldo retrotransposon peptidase (Drosophila sp.)
A02.055	RTVL-H-like putative peptidase
A02.056	human endogenous retrovirus retropepsin homologue 1
A02.057	human endogenous retrovirus retropepsin homologue 2
A02.060	type D-like endogenous retrovirus endopeptidase (Mus musculus)
A02.061	Ulysses retrotransposon peptidase (Drosophila virilis) retropepsin
A02.062	TED retrotransposon peptidase (Trichoplusia ni)
A02.063	Walleye dermal sarcoma virus retropepsin
A03.001	cauliflower mosaic virus-type endopeptidase
A03.002	bacilliform virus endopeptidase
A03.003	banana streak virus endopeptidase
A03.004	Commelina yellow mottle virus endopeptidase
A03.005	cassava vein mosaic virus-type endopeptidase
A03.006	retrotransposon peptidase (Nicotiana tabacum)
A05.001	thermopsin
A06.001	nodavirus endopeptidase
A08.001	signal peptidase II
A09.001	spumapepsin
A11.001	Copia transposon (Drosophila sp.) peptidase
A11.002	Tnt1 retrotransposon (plant) endopeptidase
A11.003	Ty1 transposon (Saccharomyces sp.) endopeptidase
A11.004	Evelknievel retrotransposon endopeptidase
A11.005	Melmoth transposon endopeptidase
A11.051	SIRE-1 (Glycine max) peptidase
A21.001	tetravirus endopeptidase
A22.001	presenilin 1
A22.002	presenilin 2
A22.003	impas 1 endopeptidase
A22.004	impas 4 endopeptidase
A22.005	impas 2 endopeptidase
A22.006	impas 5 endopeptidase
A22.007	impas 3 endopeptidase
A22.008	YKL100c protein (Saccharomyces cerevisiae)
A22.009	SEL-12 protein (Caenorhabditis elegans)

(continued)

CODE	Protease name
A22.010	hop-1 g.p. (Caenorhabditis elegans)
A24.001	type 4 prepilin peptidase 1
A24.003	type 4 prepilin peptidase 2
A24.016	preflagellin peptidase
A24.017	PibD g.p. (Sulfolobus sp.)
A26.001	omptin
A26.002	OmpP (Escherichia coli)
A26.003	plasminogen activator Pla
A26.004	protein E (Salmonella sp.)
A26.005	peptidase SopA
A9G.001	aspartic endopeptidase, plasma
A9G.008	rhodotorulapepsin
A9G.010	pycnoporopepsin
A9G.011	scytalidopepsin A
A9G.017	acid endopeptidase (Cladosporium)
A9G.018	acid endopeptidase (Paecilomyces)
A9G.019	acrocylindropepsin
A9G.020	yapsin A
C01.001	papain
C01.002	chymopapain
C01.003	caricain
C01.004	glycyl endopeptidase
C01.005	stem bromelain
C01.006	ficain
C01.007	actinidain
C01.008	asclepain
C01.009	cathepsin V
C01.010	vignain
C01.011	calotropin
C01.013	cathepsin X
C01.014	cathepsin L-like peptidase 2
C01.015	cathepsin L-like peptidase 3
C01.016	cathepsin-1
C01.017	zingipain
C01.018	cathepsin F
C01.019	CC-I endopeptidase (Carica sp.)
C01.020	CC-III endopeptidase (Carica candamarcensis)
C01.021	brassicain

(continued)

CODE	Protease name
C01.022	glycinain
C01.023	cathepsin M
C01.024	endopeptidase B (barley-type)
C01.026	ananain
C01.027	comosain
C01.028	fruit bromelain
C01.029	pseudotzain
C01.030	crustapain
C01.031	cathepsin-2
C01.032	cathepsin L
C01.033	cathepsin L-like endopeptidase (Fasciola sp.)
C01.034	cathepsin S
C01.035	cathepsin O
C01.036	cathepsin K
C01.037	cathepsin W
C01.038	cathepsin P
C01.039	cathepsin Q
C01.040	cathepsin H
C01.041	aleurain
C01.042	cathepsin R
C01.044	SmCL2-like peptidase
C01.045	cathepsin-6
C01.046	falcipain-2
C01.047	granulovirus cathepsin
C01.049	cathepsin B, plant form
C01.050	histolysain
C01.053	cathepsin-3
C01.054	2310051m13rik protein
C01.055	papain homologue (nematode)
C01.056	Rcr3 peptidase (Lycopersicon sp.)
C01.057	vinckepain-2
C01.058	peptidase similar to cathepsin 7
C01.059	peptidase similar to cathepsin 8 (Mus musculus)
C01.060	cathepsin B
C01.061	SmCB2 peptidase (Schistosoma sp.)
C01.062	cathepsin B-like endopeptidase (platyhelminth)
C01.063	falcipain-3
C01.064	RD21 endopeptidase

(continued)

CODE	Protease name
C01.065	XCP1 peptidase (Arabidopsis-type)
C01.066	cpl-1 endopeptidase
C01.067	insect 26/29 kDa peptidase
C01.068	vitellogenic cathepsin B
C01.070	dipeptidyl-peptidase I
C01.071	toxopain-1
C01.072	rhodesain
C01.073	endopeptidase 1 (mite)
C01.074	CPB endopeptidase
C01.075	cruzipain
C01.076	CPA endopeptidase
C01.077	falcipain-1
C01.079	papain homologue (Theileria-type)
C01.081	papain homologue (Dictyostelium-type)
C01.082	papain homologue (trichomonad)
C01.083	V-cath endopeptidase
C01.084	bleomycin hydrolase (animal)
C01.085	bleomycin hydrolase (yeast)
C01.086	aminopeptidase C
C01.088	oligopeptidase E
C01.089	peptidase G
C01.091	peptidase W
C01.093	miltpain
C01.094	giardain
C01.095	papain homologue (Archaeoglobus)
C01.096	melain G
C01.097	phytolacain
C01.098	CPC endopeptidase
C01.099	ervatamin B
C01.100	cruzipain 2
C01.101	cathepsin B-like peptidase, nematode
C01.102	encystation-specific endopeptidase (Giardia sp.)
C01.104	SPG31-like peptidase
C01.105	mir1 g.p. (Zea mays)
C01.107	papain homologue (Rattus norvegicus)
C01.108	peptidase similar to cathepsin 8 (Rattus norvegicus)
C01.110	similar to cathepsin M (Mus musculus)
C01.111	cathepsin Q2 (Rattus norvegicus)

(continued)

CODE	Protease name
C01.112	similar to cathepsin M (Rattus norvegicus)
C01.113	tetrain
C01.114	testin-3
C01.115	fascipain B
C01.116	ervatamin C
C01.117	senescence-associated gene 12
C01.118	allergen Blo t 1 (Blomia tropicalis)
C01.119	EhCP112 peptidase (Entamoeba histolytica)
C01.120	p48h-17 g.p. (Zinnia-type)
C01.121	XCP2 peptidase
C01.122	SERA5 peptidase (Plasmodium falciparum)
C01.123	EhCP-B peptidase (Entamoeba histolytica)
C01.124	dipeptidylpeptidase I (Plasmodium-type)
C01.125	Cwp84 g.p. (Clostridium difficile)
C02.001	calpain-1
C02.002	calpain-2
C02.003	calpain C
C02.004	calpain-3
C02.006	calpain-9
C02.007	calpain-8
C02.008	calpain-7
C02.009	calpain tra-3 (Caenorhabditis elegans)
C02.010	calpain-15
C02.011	calpain-5
C02.013	calpain-11
C02.014	calpain A
C02.015	calpain B
C02.017	calpain-12
C02.018	calpain-10
C02.019	phytocalpain
C02.020	calpain-13
C02.021	calpain-14
C02.022	Tpr peptidase (Porphyromonas gingivalis)
C02.023	calpain (Schistosoma sp.)
C03.001	poliovirus-type picornain 3C
C03.003	cowpea mosaic-type comovirus picornain 3C
C03.004	grapevine fanleaf-type nepovirus picornain 3C
C03.005	hepatitis A virus-type picornain 3C

(continued)

CODE	Protease name
C03.007	rhinovirus picornain 3C
C03.008	foot-and-mouth disease virus picornain 3C
C03.009	cardiovirus picornain 3C
C03.010	Theiler's murine encephalomyelitis virus picornain 3C
C03.011	coxsackievirus-type picornain 3C
C03.012	tomato ringspot nepovirus picornain 3C
C03.013	rhinovirus 14 3C peptidase
C03.014	human enterovirus 71 3C peptidase
C03.020	poliovirus-type picornain 2A
C03.021	rhinovirus picornain 2A
C03.022	coxsackievirus-type picornain 2A
C03.023	parechovirus picornain 3C
C03.024	rice tungro spherical virus-type endopeptidase
C03.025	tomato black ring virus-type picornain
C04.001	nuclear-inclusion-a endopeptidase (plum pox virus)
C04.002	potato virus Y-type NIa endopeptidase
C04.003	tobacco vein mottling virus-type NIa endopeptidase
C04.004	tobacco etch virus NIa endopeptidase
C04.005	Ornithogalum mosaic virus NIa endopeptidase
C04.006	yam mosaic virus NIa endopeptidase
C04.007	shallot potyvirus NIa endopeptidase
C04.008	bean yellow mosaic virus-type NIa endopeptidase
C04.009	papaya ringspot virus NIa endopeptidase
C04.010	pea seed-borne mosaic virus NIa endopeptidase
C04.011	Johnson grass mosaic virus NIa endopeptidase
C04.012	rye grass mosaic virus NIa endopeptidase
C04.013	sweet potato mild mottle virus NIa endopeptidase
C04.014	potato virus A NIa endopeptidase
C05.001	adenain
C06.001	potato virus Y-type helper component peptidase
C06.002	barley yellow mosaic virus-type helper component peptidase
C07.001	chestnut blight fungus virus p29 peptidase
C08.001	chestnut blight fungus virus p48 peptidase
C09.001	sindbis virus-type nsP2 peptidase
C10.001	streptopain
C10.002	PrtT peptidase
C10.003	periodontain
C11.001	clostripain

(continued)

CODE	Protease name
C12.001	ubiquitinyl hydrolase-L1 (mammal)
C12.002	ubiquitinyl hydrolase-YUH1
C12.003	ubiquitinyl hydrolase-L3
C12.004	ubiquitinyl hydrolase-BAP1
C12.005	ubiquitinyl hydrolase-UCH37
C12.006	ubiquitinyl hydrolase B40085
C12.007	ubiquitinyl hydrolase isozyme L4 (Mus musculus)
C12.008	ubiquitinyl hydrolase UCH-D (Drosophila melanogaster)
C12.009	Uch2 peptidase (Schizosaccharomyces pombe)
C13.001	legumain (plant beta form)
C13.002	legumain (plant alpha form)
C13.003	legumain (non-chordate)
C13.004	legumain (chordate)
C13.005	glycosylphosphatidylinositol: protein transamidase
C13.006	legumain (plant gamma form)
C14.001	caspase-1
C14.002	CED-3 endopeptidase
C14.003	caspase-3
C14.004	caspase-7
C14.005	caspase-6
C14.006	caspase-2
C14.007	caspase-4
C14.008	caspase-5
C14.009	caspase-8
C14.010	caspase-9
C14.011	caspase-10
C14.012	caspase-11
C14.013	caspase-12
C14.015	caspase (insect 1)
C14.016	caspase (insect 2)
C14.017	caspase-13
C14.018	caspase-14
C14.019	caspase DRONC (Drosophila melanogaster)
C14.021	ICEY peptidase
C14.023	STRICA g.p. (Drosophila melanogaster)
C14.025	caspase DAMM (Drosophila melanogaster)
C14.026	paracaspase
C14.030	Caspy g.p. (Danio rerio)

(continued)

CODE	Protease name
C14.031	Caspy2 g.p. (Danio rerio)
C14.032	putative caspase (Homo sapiens)
C14.033	metacaspase-4 (Arabidopsis thaliana)
C14.034	metacaspase-9 (Arabidopsis thaliana)
C14.035	yeast metacaspase-1
C15.001	pyroglutamyl-peptidase I (prokaryote)
C15.010	pyroglutamyl-peptidase I (eukaryote)
C16.001	murine hepatitis coronavirus papain-like endopeptidase 1
C16.002	human coronavirus 229E papain-like endopeptidase 1
C16.003	porcine epidemic diarrhea virus papain-like endopeptidase 1
C16.004	porcine transmissible gastroenteritis coronavirusvirus papain-like endopeptidase 1
C16.005	avian infectious bronchitis coronavirus papain-like endopeptidase 1
C16.006	murine hepatitis coronavirus papain-like endopeptidase 2
C16.008	porcine transmissible gastroenteritis coronavirus papain-like endopeptidase 2
C16.009	SARS coronavirus papain-like endopeptidase
C18.001	hepatitis C virus endopeptidase 2
C19.001	ubiquitin-specific peptidase 5
C19.002	Ubp1 ubiquitin peptidase
C19.003	Ubp2 ubiquitin peptidase
C19.004	Ubp3 ubiquitin peptidase
C19.005	Doa4 ubiquitin peptidase
C19.006	Ubp5 ubiquitin peptidase
C19.007	Fat facets protein
C19.008	ubiquitin-specific peptidase (plant)
C19.009	ubiquitin-specific peptidase 6
C19.010	ubiquitin-specific peptidase 4
C19.011	ubiquitin-specific peptidase 8
C19.012	ubiquitin-specific peptidase 13
C19.013	ubiquitin-specific peptidase 2
C19.014	ubiquitin-specific peptidase 11
C19.015	ubiquitin-specific peptidase 14
C19.016	ubiquitin-specific peptidase 7
C19.017	ubiquitin-specific peptidase 9X
C19.018	ubiquitin-specific peptidase 10
C19.019	ubiquitin-specific peptidase 1
C19.020	ubiquitin-specific peptidase 12
C19.021	ubiquitin-specific peptidase 16
C19.022	ubiquitin-specific peptidase 15

(continued)

CODE	Protease name
C19.023	ubiquitin-specific peptidase 17
C19.024	ubiquitin-specific peptidase 19
C19.025	ubiquitin-specific peptidase 20
C19.026	ubiquitin-specific peptidase 3
C19.028	ubiquitin-specific peptidase 9Y
C19.030	ubiquitin-specific peptidase 18
C19.031	DUB-1 ubiquitin-specific peptidase
C19.032	DUB-2 ubiquitin-specific peptidase
C19.034	ubiquitin-specific peptidase 21
C19.035	ubiquitin-specific peptidase 22
C19.037	ubiquitin-specific peptidase 33
C19.040	ubiquitin-specific peptidase 29
C19.041	ubiquitin-specific peptidase 25
C19.042	ubiquitin-specific peptidase 36
C19.044	ubiquitin-specific peptidase 32
C19.045	ubiquitin-specific peptidase 26 (mouse-type)
C19.046	ubiquitin-specific peptidase 26 (human-type)
C19.047	ubiquitin-specific peptidase 24
C19.048	ubiquitin-specific peptidase 42
C19.051	Usp9y g.p. (Mus musculus)
C19.052	ubiquitin-specific peptidase 46
C19.053	ubiquitin-specific peptidase 37
C19.054	ubiquitin-specific peptidase 28
C19.055	ubiquitin-specific peptidase 47
C19.056	ubiquitin-specific peptidase 38
C19.057	ubiquitin-specific peptidase 44
C19.058	ubiquitin-specific peptidase 50
C19.059	ubiquitin-specific peptidase 35
C19.060	ubiquitin-specific peptidase 30
C19.064	ubiquitin-specific peptidase 45
C19.065	ubiquitin-specific peptidase 51
C19.067	ubiquitin-specific peptidase 34
C19.068	ubiquitin-specific peptidase 48
C19.069	ubiquitin-specific peptidase 40
C19.071	ubiquitin-specific peptidase 31
C19.073	ubiquitin-specific peptidase 49
C19.076	protein similar to high mobility group protein
C19.077	Dub5 peptidase (Mus musculus)

(continued)

CODE	Protease name
C19.078	USP17-like peptidase
C19.079	ubiquitin-specific peptidase 6 (Saccharomyces cerevisiae)
C19.080	ubiquitin-specific peptidase 54
C19.081	ubiquitin-specific peptidase 53
C19.082	deubiquitinating enzyme 6 (Mus musculus)
C19.083	deubiquitinating enzyme 14 (Saccharomyces cerevisiae)
C19.084	deubiquitinating enzyme 14 (plant)
C19.085	DUB-1A peptidase (Mus musculus)
C19.086	DUB-2A peptidase (Mus musculus)
C19.087	ubiquitin-specific peptidase 8 (Saccharomyces cerevisiae)
C19.088	ubiquitin-specific peptidase 10 (Saccharomyces cerevisiae)
C21.001	tymovirus endopeptidase
C23.001	carlavirus endopeptidase
C24.001	rabbit hemorrhagic disease virus 3C-like endopeptidase
C24.002	feline calicivirus 3C-like endopeptidase
C25.001	gingipain R
C25.002	gingipain K
C25.003	gingipain R2
C26.001	gamma-glutamyl hydrolase
C27.001	rubella virus endopeptidase
C28.001	foot-and-mouth disease virus L-peptidase
C28.002	equine rhinovirus L-peptidase
C30.001	hepatitis coronavirus picornain 3C-like endopeptidase
C30.002	avian infectious bronchitis coronavirus 3C-like endopeptidase
C30.003	human coronavirus 229E main endopeptidase
C30.004	porcine transmissible gastroenteritis virus-type main endopeptidase
C30.005	SARS coronavirus picornain 3C-like endopeptidase
C31.001	porcine respiratory and reproductive syndrome arterivirus-type cysteine peptidase alpha
C32.001	equine arteritis virus-type cysteine peptidase
C33.001	equine arterivirus Nsp2-type cysteine peptidase
C36.001	beet necrotic yellow vein furovirus-type papain-like endopeptidase
C37.001	calicivirin
C39.001	bacteriocin-processing peptidase
C39.003	streptococcin SA-FF22 processing peptidase (Streptococcus pyogenes)
C39.004	mersacidin lantibiotic processing peptidase (Bacillus sp.)
C39.005	colicin V processing peptidase
C39.006	mutacin II processing peptidase (Streptococcus mutans)
C39.007	lacticin 481 processing peptidase (Lactococcus lactis)

(continued)

CODE	Protease name
C40.001	dipeptidyl-peptidase VI (bacteria)
C40.002	murein endopeptidase lytF (Bacillus sp.)
C40.003	lytE g.p. (Bacillus-type)
C40.004	spr g.p. (Escherichia-type)
C42.001	beet yellows virus-type papain-like peptidase
C42.002	papain-like peptidase 2 (citrus tristeza virus)
C42.003	L1 peptidase (citrus tristeza virus)
C44.001	amidophosphoribosyltransferase precursor
C45.001	acyl-coenzyme A:6-aminopenicillanic acid acyl-transferase precursor
C46.001	hedgehog protein
C46.002	Sonic hedgehog protein
C46.003	Indian hedgehog protein
C46.004	Desert hedgehog protein
C46.005	Tiggy-winkle protein
C47.001	staphopain A
C47.002	staphopain B
C47.003	ecp g.p. (Staphylococcus epidermidis)
C48.001	Ulp1 endopeptidase
C48.002	SENP1 peptidase
C48.003	SENP3 peptidase
C48.004	SENP6 peptidase
C48.005	Ulp2 endopeptidase
C48.007	SENP2 peptidase
C48.008	SENP5 peptidase
C48.009	SENP7 peptidase
C48.011	SENP8 peptidase
C48.012	SENP4 peptidase
C48.018	peptidase similar to SUMO-1-specific peptidase (Rattus horvegicus)
C48.020	LOC297623 peptidase (Rattus norvegicus)
C48.021	similar to SUMO-1-specific peptidase (Mus musculus)
C48.022	esd4 g.p. (Arabidopsis thaliana)
C48.023	XopD peptidase
C48.024	Ulp1 g.p. (Drosophila melanogaster)
C50.001	separase
C51.001	D-alanyl-glycyl endopeptidase (staphylococcal phage phi11)
C53.001	pestivirus Npro endopeptidase
C54.001	ATG4 peptidase (Saccharomyces cerevisiae)
C54.002	autophagin-2

(continued)

CODE	Protease name
C54.003	autophagin-1
C54.004	autophagin-3
C54.005	autophagin-4
C55.001	YopJ endopeptidase
C55.003	AvrA g.p. (Salmonella sp.)
C55.004	PopP1 g.p. (Ralstonia solanacearum)
C55.005	AvrPpiG1 g.p. (Pseudomonas syringae)
C55.006	AvrXv4 (Xanthomonas campestris)
C55.007	VopA g.p. (Vibrio parahaemolyticus)
C56.001	Pfpl endopeptidase
C56.002	DJ-1 putative peptidase
C56.004	YDR533C peptidase
C56.006	Hsp31 g.p. (Escherichia coli)
C57.001	vaccinia virus I7L processing peptidase
C58.001	YopT peptidase (Yersinia-type)
C58.002	AvrPphB g.p. (Pseudomonas syringae)
C59.001	penicillin V acylase (Bacillus-type)
C60.001	sortase A
C60.002	sortase B
C60.003	sortase C2
C62.001	gill-associated virus 3C-like peptidase
C63.001	African swine fever virus processing peptidase
C64.001	Cezanne deubiquitinating peptidase
C64.002	Cezanne-2 peptidase
C64.003	tumor necrosis factor alpha-induced protein 3
C64.004	TRABID protein
C65.001	otubain-1
C65.002	otubain-2
C65.003	otubain-3
C66.001	IdeS peptidase (Streptococcus pyogenes)
C67.001	CylD protein
C69.001	dipeptidase A
C69.002	arginine aminopeptidase (Streptococcus sp.)
C70.001	AvrRpt2 g.p. (Pseudomonas syringae)
C71.001	pseudomurein endoisopeptidase Pei
C72.001	HopPtoN g.p. (Pseudomonas syringae)
C9B.001	lysosomal dipeptidase II
C9C.001	dipeptidyl-dipeptidase

(continued)

CODE	Protease name
C9G.001	cathepsin N
C9G.002	leucoegresin-generating endopeptidase
C9G.003	ATP-dependent cysteine endopeptidase
C9G.004	mitochondrial cysteine endopeptidase
C9G.005	cathepsin T
C9G.006	nuclear cysteine endopeptidase
C9G.009	cathepsin M (old)
C9G.012	cancer procoagulant
C9G.013	prohormone thiol peptidase
C9G.020	archaean cysteine endopeptidase
C9G.021	lobster muscle calpain-like peptidase
C9G.022	cysteine endopeptidase (Micrococcus sp. INIA 528)
C9G.024	alanyl aminopeptidase (cysteine type) (Pseudomonas aeruginosa)
C9G.025	cysteine peptidase 1 (Vibrio harveyi)
C9G.026	avian infectious bronchitis coronavirus papain-like endopeptidase 2
G01.001	scytalidoglutamic peptidase
G01.002	aspergilloglutamic peptidase
G01.003	endopeptidase EapB
G01.004	endopeptidase EapC
M01.001	aminopeptidase N
M01.002	lysyl aminopeptidase (bacteria)
M01.003	aminopeptidase A
M01.004	leukotriene A4 hydrolase
M01.005	alanyl aminopeptidase (proteobacteria)
M01.006	Ape2 aminopeptidase
M01.007	Aap1' aminopeptidase
M01.008	pyroglutamyl-peptidase II
M01.009	aminopeptidase N (actinomycete-type)
M01.010	cytosol alanyl aminopeptidase
M01.011	cystinyl aminopeptidase
M01.012	aminopeptidase G (Streptomyces sp.)
M01.013	aminopeptidase N (insect)
M01.014	aminopeptidase B
M01.015	aminopeptidase H11 (nematode)
M01.016	aminopeptidase Ey
M01.017	Yin7 g.p. (Saccharomyces cerevisiae)
M01.018	aminopeptidase PILS
M01.020	tricorn interacting factor F2 (Thermoplasma sp.)

(continued)

CODE	Protease name
M01.021	tricorn interacting factor F3 (Thermoplasma sp.)
M01.024	leukocyte-derived arginine aminopeptidase
M01.025	aminopeptidase-1 (Caenorhabditis elegans)
M01.027	laeverin
M01.028	aminopeptidase O
M01.029	PfA-M1 aminopeptidase (Plasmodium falciparum-type)
M02.001	angiotensin-converting enzyme peptidase unit 1
M02.002	peptidyl-dipeptidase Acer
M02.003	peptidyl-dipeptidase Ance
M02.004	angiotensin-converting enzyme peptidase unit 2
M02.005	peptidyl-dipeptidase A (Theromyzon)
M02.006	angiotensin-converting enzyme 2
M03.001	thimet oligopeptidase
M03.002	neurolysin
M03.003	saccharolysin
M03.004	oligopeptidase A
M03.005	peptidyl-dipeptidase Dcp
M03.006	mitochondrial intermediate peptidase
M03.007	oligopeptidase F
M03.009	oligopeptidase MepB
M04.001	thermolysin
M04.003	vibriolysin
M04.005	pseudolysin
M04.006	Msp peptidase (Legionella sp.)
M04.007	coccolysin
M04.008	thermolysin homologue (Listeria sp.)
M04.009	aureolysin
M04.010	vimelysin (Vibrio str. T1800)
M04.011	lambda toxin (Clostridium sp.)
M04.012	neutral peptidase B (Bacillus sp.)
M04.014	bacillolysin
M04.016	PA peptidase (Aeromonas-type)
M04.017	griselysin
M04.018	stearolysin
M04.019	MprIII (Alteromonas sp. strain O-7)
M04.020	pap6 endopeptidase
M04.021	neutral endopeptidase (Thermoactinomyces sp. 27a)
M05.001	mycolysin

(continued)

CODE	Protease name
M06.001	immune inhibitor A (Bacillus sp.)
M06.004	inhA2 g.p. (Bacillus sp.)
M07.001	snapalysin
M08.001	leishmanolysin
M08.002	invadolysin
M08.003	leishmanolysin-2
M09.001	microbial collagenase (Vibrio sp.)
M09.002	collagenase colA
M09.003	collagenase colH
M09.004	endopeptidase VMC (Vibrio sp.)
M10.001	collagenase 1
M10.002	collagenase 2
M10.003	gelatinase A
M10.004	gelatinase B
M10.005	stromelysin 1
M10.006	stromelysin 2
M10.007	stromelysin 3
M10.008	matrilysin
M10.009	macrophage elastase
M10.010	envelysin
M10.012	plant matrixin
M10.013	collagenase 3
M10.014	membrane-type matrix metallopeptidase 1
M10.015	membrane-type matrix metallopeptidase 2
M10.016	membrane-type matrix metallopeptidase 3
M10.017	membrane-type matrix metallopeptidase 4
M10.018	collagenase 4
M10.019	enamelysin
M10.020	fragilysin
M10.021	matrix metallopeptidase 19
M10.022	matrix metallopeptidase 23B
M10.023	membrane-type matrix metallopeptidase 5
M10.024	membrane-type matrix metallopeptidase 6
M10.025	HMMP peptidase (Hydra vulgaris)
M10.026	matrix metallopeptidase 21
M10.027	matrix metallopeptidase 22
M10.029	matrilysin-2
M10.030	epilysin

(continued)

CODE	Protease name
M10.031	Dm1 matrix matallopeptidase (Diptera)
M10.032	matrixin V
M10.033	collagenase-like A peptidase (rodent)
M10.034	collagenase-like B peptidase (rodent)
M10.035	S-layer-associated peptidase (Caulobacter crescentus)
M10.036	Dm2-MMP peptidase (Drosophila melanogaster)
M10.037	matrix metallopeptidase 23A
M10.051	serralysin
M10.052	peptidase A (Erwinia-type)
M10.053	peptidase B (Erwinia-type)
M10.054	peptidase C (Erwinia-type)
M10.055	peptidase G (Erwinia-type)
M10.056	aeruginolysin
M10.057	mirabilysin
M10.060	epralysin
M10.062	psychrophilic alkaline metallopeptidase (Pseudomonas sp.)
M11.001	gametolysin
M11.002	VMP peptidase (Volvox carteri)
M11.003	mmp2 g.p. (Chlamydomonas reinhardtii)
M12.001	astacin
M12.002	meprin alpha subunit
M12.003	myosinase
M12.004	meprin beta subunit
M12.005	procollagen C-peptidase
M12.006	choriolysin L
M12.007	choriolysin H
M12.008	nephrosin
M12.010	tolloid
M12.011	tolkin
M12.013	SpAN g.p. (Strongylocentrotus purpuratus)
M12.014	hatching enzyme (Xenopus)
M12.015	xolloid
M12.016	mammalian tolloid-like 1 protein
M12.017	metallopeptidase 1 (Hydra)
M12.018	mammalian tolloid-like 2 protein
M12.019	MIG-17 endopeptidase (Caenorhabditis elegans)
M12.020	ADAM28 endopeptidase (mouse-type)
M12.021	ADAMTS9 endopeptidase

(continued)

CODE	Protease name
M12.022	brevilysin H6
M12.024	ADAMTS14 endopeptidase
M12.025	ADAMTS15 endopeptidase
M12.026	ADAMTS16 endopeptidase
M12.027	ADAMTS17 endopeptidase
M12.028	ADAMTS18 endopeptidase
M12.029	ADAMTS19 endopeptidase
M12.030	peptidase similar to ADAMTS-1 endopeptidase (Mus musculus)
M12.031	peptidase similar to ADAMTS-9 endopeptidase (Rattus norvegicus)
M12.066	flavastacin
M12.131	acutolysin A
M12.132	bilitoxin (Agkistrodon bilineatus)
M12.133	fibrolase (Agkistrodon contortrix)
M12.134	halylysin a
M12.135	gon-1 g.p. (Caenorhabditis elegans)
M12.136	leucolysin
M12.137	BHRa hemorrhagin (Bitis arietans)
M12.138	jararhagin
M12.139	bothrolysin
M12.140	bothropasin
M12.141	adamalysin
M12.142	atrolysin A
M12.143	atrolysin B
M12.144	atrolysin C
M12.145	atrolysin E
M12.146	atrolysin F
M12.147	atroxase
M12.148	basilysin
M12.149	horrilysin
M12.150	ruberlysin
M12.151	ecarin
M12.152	ophiolysin
M12.153	fibrinolytic endopeptidase (Philodryas olfershii)
M12.154	trimerelysin I
M12.155	trimerelysin II
M12.156	trimerelysin IIA
M12.157	mucrolysin
M12.158	russellysin

(continued)

CODE	Protease name
M12.159	cobrin
M12.160	venom metalloendopeptidase PREH
M12.161	kistomin (Calloselasma rhodostoma)
M12.162	mutalysin II
M12.163	graminelysin (Trimeresurus gramineus)
M12.164	lebetase
M12.166	BaH1 endopeptidase (Bothrops asper)
M12.167	najalysin
M12.168	alpha peptidase (Crotalus atrox)
M12.169	metalloendopeptidase (Bothrops moojeni)
M12.170	jararafibrase II (Bothrops jararaca)
M12.171	HT-1 endopeptidase (Crotalus viridis)
M12.172	carinactivase
M12.173	mocarhagin
M12.176	fibrinolytic peptidase M5 (Crotalus molossus)
M12.177	multactivase
M12.178	brevilysin L6
M12.179	bilitoxin 2 (Agkistrodon bilineatus)
M12.180	Mde10 metalloendopeptidase (Schizosaccharomyces)
M12.184	mutalysin I
M12.185	moojeni peptidase B
M12.186	vascular apoptosis-inducing protein 1
M12.187	similar to ADAM 21 preproprotein (Rattus norvegicus)
M12.188	ADAMTS20 endopeptidase (Mus musculus)
M12.201	ADAM1 endopeptidase
M12.202	AdamlA g.p. (Mus musculus)
M12.203	AdamlB g.p. (Mus musculus)
M12.208	ADAM8 endopeptidase
M12.209	ADAM9 endopeptidase
M12.210	ADAM10 endopeptidase
M12.211	Kuzbanian protein (non-mammalian)
M12.212	ADAM12 endopeptidase
M12.213	ADAM13 endopeptidase
M12.214	adamalysin-19
M12.215	ADAM15 endopeptidase
M12.217	ADAM 17 endopeptidase
M12.218	ADAM20 endopeptidase
M12.219	ADAMDEC1 endopeptidase

(continued)

CODE	Protease name
M12.220	ADAMTS3 endopeptidase
M12.221	ADAMTS4 endopeptidase
M12.222	ADAMTS1 endopeptidase
M12.224	ADAM28 endopeptidase (human-type)
M12.225	ADAMTS5 endopeptidase
M12.226	ADAMTS8 endopeptidase
M12.227	ADAM24 endopeptidase
M12.228	ADAM25 endopeptidase
M12.229	ADAM26 endopeptidase
M12.230	ADAMTS6 endopeptidase
M12.231	ADAMTS7 endopeptidase
M12.232	ADAM30 endopeptidase
M12.233	ADAM31 endopeptidase (rodent)
M12.234	ADAM21 endopeptidase (Homo sapiens)
M12.235	ADAMTS10 endopeptidase
M12.236	kaouthiagin
M12.237	ADAMTS12 endopeptidase
M12.238	membrane-anchored metallopeptidase (Xenopus laevis)
M12.241	ADAMTS13 endopeptidase
M12.242	TM-3 peptidase (Trimeresurus mucrosquamatus)
M12.243	testase 4 (Mus musculus)
M12.244	ADAM33 endopeptidase
M12.245	ovastacin
M12.246	ADAMTS20 endopeptidase (Homo sapiens)
M12.247	peptidase similar to ADAM 21 endopeptidase (Mus musculus)
M12.248	peptidase similar to ADAMTS-6 endopeptidase (Mus musculus)
M12.249	testase-7
M12.250	testase-6
M12.251	testase-8
M12.252	testase-9
M12.301	procollagen I N-endopeptidase
M12.302	ADAMTS adt-1 endopeptidase (Caenorhabditis elegans)
M12.303	acutolysin C
M12.304	jararafibrase III (Bothrops jararaca)
M12.305	jararafibrase IV (Bothrops jararaca)
M12.306	BHRb haemorrhagin (Bitis arietans)
M12.307	halylysin b
M12.308	halylysin c

(continued)

CODE	Protease name
M12.309	hemorrhagic toxin I (Gloydius halys blomhoffii)
M12.310	metallopeptidase MTP-1 (Ancylostoma caninum)
M12.311	BaP1 endopeptidase (Bothrops asper)
M12.312	neuwiedase (Bothrops neuwiedi)
M12.313	jerdonitin (Trimeresurus jerdonii)
M12.314	EBR1 peptidase (Strongylocentrotus sp.)
M12.315	halysase (Gloydius halys)
M12.316	triflamp (Trimeresurus flavoviridis)
M12.317	acutolysin D
M12.318	C17G1.6 g.p. (Caenorhabditis elegans)
M12.319	C26C6.3 gene (Caenorhabditis elegans)
M12.321	TOH-2 g.p. (Caenorhabditis elegans)
M13.001	neprilysin
M13.002	endothelin-converting enzyme 1
M13.003	endothelin-converting enzyme 2
M13.004	oligopeptidase O1
M13.005	oligopeptidase O3
M13.007	DINE peptidase
M13.008	neprilysin-2
M13.009	PgPepO oligopeptidase
M13.010	oligopeptidase O2
M13.011	nematode neprilysin homologue
M13.012	Nep2 peptidase (Drosophila melanogaster)
M13.090	Kell blood-group protein
M13.091	PHEX endopeptidase
M14.001	carboxypeptidase A1
M14.002	carboxypeptidase A2
M14.003	carboxypeptidase B
M 14.004	carboxypeptidase N
M14.005	carboxypeptidase E
M14.006	carboxypeptidase M
M14.007	carboxypeptidase T
M14.008	gamma-D-glutamyl-(L)-meso-diaminopimelate peptidase I
M14.009	carboxypeptidase U
M14.010	carboxypeptidase A3
M14.011	metallocarboxypeptidase D peptidase unit 1
M 14.012	metallocarboxypeptidase Z
M14.014	carboxypeptidase MeCPA

(continued)

CODE	Protease name
M14.016	metallocarboxypeptidase D peptidase unit 2
M14.017	carboxypeptidase A4
M14.018	carboxypeptidase A6
M14.020	carboxypeptidase A5
M14.021	metallocarboxypeptidase O
M 14.023	CPG70 carboxypeptidase (Porphyromonas gingivalis)
M14.024	insect gut carboxypeptidase-1
M14.027	hypothetical protein flj14442 (Homo sapiens)
M14.029	A430081C19RIK protein (Mus musculus)
M14.030	hypothetical Zn-dependent exopeptidase (Mus musculus)
M14.031	insect gut carboxypeptidase-2
M15.001	zinc D-Ala-D-Ala carboxypeptidase (Streptomyces sp.)
M15.002	DD-carboxypeptidase pdcA (Myxococcus xanthus)
M15.003	van XYc peptidase
M15.010	vanY D-Ala-D-Ala carboxypeptidase
M15.011	vanX D-Ala-D-Ala dipeptidase
M15.020	ply endolysin
M16.001	pitrilysin
M16.002	insulysin
M16.003	mitochondrial processing peptidase beta-subunit
M16.004	chloroplast (stromal) processing peptidase
M16.005	nardilysin
M16.006	pqqF protein
M 16.007	Axl1 peptidase
M 16.008	Ste23 peptidase
M16.009	eupitrilysin
M16.011	falcilysin
M16.012	PreP peptidase
M16.013	CYM1 peptidase (Saccharomyces cerevisiae)
M17.001	leucyl aminopeptidase (animal)
M17.002	leucyl aminopeptidase (plant)
M17.003	aminopeptidase A (bacteria)
M17.004	PepB aminopeptidase
M18.001	aminopeptidase I
M18.002	aspartyl aminopeptidase
M19.001	membrane dipeptidase
M19.003	dipeptidase AC
M19.007	thermostable dipeptidase (Brevibacillus-type)

(continued)

CODE	Protease name
M20.001	glutamate carboxypeptidase
M20.002	Gly-X carboxypeptidase
M20.003	peptidase T
M20.004	peptidase V
M20.005	cytosolic nonspecific dipeptidase
M20.006	carnosinase
M20.007	Xaa-His dipeptidase
M20.008	carboxypeptidase Ss1
M20.010	DapE peptidase
M20.012	Pep581 peptidase (Prevotella albensis)
M22.001	O-sialoglycoprotein endopeptidase
M22.002	yeaZ protein
M22.005	Pgp1 peptidase
M23.001	beta-lytic metalloendopeptidase (myxobacteria)
M23.002	staphylolysin
M23.003	fibrinolytic endopeptidase (Aeromononas)
M23.004	lysostaphin
M23.005	zoocin A
M23.006	YibP peptidase
M23.007	enterolysin A
M24.001	methionyl aminopeptidase 1
M24.002	methionyl aminopeptidase 2
M24.003	Xaa-Pro dipeptidase (bacteria)
M24.004	aminopeptidase P (bacteria)
M24.005	aminopeptidase P2
M24.007	Xaa-Pro dipeptidase (eukaryote)
M24.008	Xaa-Pro dipeptidase (archaea)
M24.009	aminopeptidase P1
M24.026	aminopeptidase P homologue
M24.031	leucine aminopeptidase (Thermotoga maritima)
M26.001	IgA1-specific metalloendopeptidase
M26.002	ZmpB metallopeptidase (Streptococcus sp.)
M26.003	ZmpC metallopeptidase (Streptococcus pneumoniae)
M27.001	tentoxilysin
M27.002	bontoxilysin
M28.001	aminopeptidase Y
M28.002	aminopeptidase Ap1
M28.003	aminopeptidase S

(continued)

CODE	Protease name
M28.004	aminopeptidase apAC (Aeromonas caviae)
M28.005	IAP aminopeptidase
M28.007	AMP1 putative carboxypeptidase
M28.008	PA2939 g.p. (Pseudomonas aeruginosa)
M28.010	glutamate carboxypeptidase II
M28.011	NAALADASE L peptidase
M28.012	glutamate carboxypeptidase III
M28.014	plasma glutamate carboxypeptidase
M28.015	aminopeptidase ES-62 (Acanthocheilonema viteae)
M28.019	aminopeptidase SSAP (Streptomyces septatus)
M29.001	aminopeptidase T
M29.002	aminopeptidase II (Bacillus-type)
M29.004	PepS aminopeptidase
M30.001	hyicolysin
M32.001	carboxypeptidase Taq
M32.002	carboxypeptidase Pfu
M34.001	anthrax lethal factor
M35.001	penicillolysin
M35.002	deuterolysin
M35.003	extracellular endopeptidase (Aeromonas-type)
M35.004	peptidyl-Lys metalloendopeptidase
M36.001	fungalysin
M38.001	beta-aspartyl dipeptidase
M38.002	Pro-Hyp dipeptidase
M41.001	FtsH endopeptidase
M41.002	Afg3 g.p. (Saccharomyces cerevisiae)
M41.003	m-AAA peptidase
M41.004	i-AAA peptidase
M41.005	FtsH endopeptidase homologue, chloroplast
M41.006	paraplegin
M41.007	Afg3-like protein 2
M41.009	FtsH-2 peptidase
M41.010	Afg3-like protein 1
M41.016	ATP-dependent zinc metallopeptidase (Mus musculus)
M42.001	glutamyl aminopeptidase (bacterium)
M42.002	bacillus aminopeptidase I (Geobacillus/Bacillus stearothermophilus)
M42.003	PTET aminopeptidase (Pyrococcus sp.)
M42.004	PTET2 aminopeptidase (Pyrococcus sp.)

(continued)

CODE	Protease name
M42.005	TET aminopeptidase (Halobacterium sp.)
M43.001	cytophagalysin
M43.002	metallopeptidase MEP1 (Metarhizium)
M43.004	pappalysin-1
M43.005	pappalysin-2
M44.001	pox virus metalloendopeptidase
M48.001	Ste24 endopeptidase
M48.002	HtpX endopeptidase
M48.003	farnesylated-protein converting enzyme 1
M48.004	HtpX-2 endopeptidase
M48.009	YhfN protein (Bacillus sp.)
M48.010	PAB0555 protein (Pyrococcus abyssi)
M48.011	small heat-shock protein (Plasmodium vivax)
M48.018	Oma1 endopeptidase (Saccharomyces cerevisiae)
M49.001	dipeptidyl-peptidase III
M50.001	S2P peptidase
M50.002	sporulation factor SpoIVFB
M50.003	YUP8H12.25 protein (Arabidopsis thaliana)
M50.004	RseP peptidase
M52.001	HybD endopeptidase
M52.002	HyaD endopeptidase
M52.003	HycI endopeptidase
M55.001	D-aminopeptidase DppA
M56.001	BlaR1 peptidase
M56.002	MecR1 g.p. (Staphylococcus sp.)
M56.003	PenR1 g.p. (Bacillus licheniformis)
M57.001	prtB g.p. (Myxococcus xanthus)
M60.001	enhancin
M61.001	glycyl aminopeptidase
M63.001	gpr peptidase
M64.001	IgA peptidase (Clostridium ramosum)
M66.001	StcE peptidase
M67.001	Poh1 peptidase
M67.002	Jab1/MPN domain metalloenzyme
M67.006	AMSH deubiquitinating peptidase
M67.007	C6.1A-like putative peptidase
M67.008	putative peptidase (Homo sapiens chromosome 2)
M67.010	JAMM-like protein (Archaeoglobus-type)

(continued)

CODE	Protease name
M72.001	peptidyl-Asp metalloendopeptidase
M73.001	camelysin
M74.001	murein endopeptidase
M75.001	imelysin
M9A.002	tripeptide aminopeptidase
M9A.005	clostridial aminopeptidase
M9A.007	Xaa-Trp aminopeptidase
M9A.008	tryptophanyl aminopeptidase
M9A.009	aminopeptidase X
M9A.010	aminopeptidase yscCo-II
M9A.011	neuron-specific aminopeptidase
M9A.012	glycyl aminopeptidase (Actinomucor elegans)
M9B.001	Xaa-Arg dipeptidase
M9B.004	Met-Xaa dipeptidase
M9D.001	peptidyl-dipeptidase B
M9D.002	proline-specific peptidyl-dipeptidase (Streptomyces)
M9E.002	alanine carboxypeptidase
M9E.003	mitochondrial carboxypeptidase
M9E.004	membrane Pro-X carboxypeptidase
M9E.007	carboxypeptidase G3
M9G.003	acrolysin
M9G.005	succinyl-tri-alanyl-p-nitroaniline hydrolase
M9G.008	plant metalloendopeptidase
M9G.009	neutral endopeptidase (Aspergillus oryzae)
M9G.018	neutral endopeptidase (Micrococcus caseolyticus)
M9G.021	metalloendopeptidase QG (Escherichia coli)
M9G.022	peptidase Ci (Escherichia coli)
M9G.025	magnolysin
M9G.026	dactylysin
M9G.028	magaininase
M9G.029	MAP1 peptidase (Myxococcus xanthus)
M9G.030	dynorphin-processing endopeptidase (metallo-type)
M9G.031	cyclic peptidase (Lactocobacillus)
M9G.034	metallopeptidase ShpII (Staphylococcus hyicus)
M9G.035	endopeptidase ECP 32 (Escherichia coli)
M9G.036	gonadotropin beta-subunit nicking enzyme
M9G.037	dithiothreitol-sensitive tetrameric peptidase
M9G.039	procollagen II N-peptidase

(continued)

CODE	Protease name
M9G.040	hepatitis B virus binding factor
M9G.041	aharin
M9G.043	collagenase (Empedobacter collagenolyticum)
M9G.044	endopeptidase Thr-N
M9G.047	insulin-cleaving periplasmic peptidase (Acinetobacter calcoaceticus)
M9G.049	procollagen III N-peptidase
M9G.051	ZPA-processing enzyme
S01.001	chymotrypsin A (cattle-type)
S01.003	mast cell peptidase 2 (Mus musculus)
S01.004	Cma2 g.p. (Mus musculus)
S01.005	mast cell peptidase 4 (Rattus)
S01.008	mast cell peptidase 10 (Rattus)
S01.009	mast cell peptidase 8 (Rattus)
S01.010	granzyme B, human-type
S01.011	testisin
S01.012	mast cell peptidase 3 (Rattus)
S01.013	Nudel peptidase
S01.015	tryptase beta (Homo sapiens)
S01.017	kallikrein hK5
S01.018	scolexin
S01.019	corin
S01.020	kallikrein hK12
S01.021	DESC1 peptidase
S01.022	ovotryptase (Xenopus laevis)
S01.023	flavoxobin
S01.024	ovotryptase 2 (Xenopus laevis)
S01.025	mast cell peptidase 6 (mouse numbering)
S01.026	mast cell peptidase 7 (mouse numbering)
S01.028	tryptase gamma 1
S01.029	kallikrein hK14
S01.030	granzyme N
S01.031	peptidase 9 (Dermatophagoides-type)
S01.033	hyaluronan-binding peptidase
S01.034	transmembrane peptidase, serine 4
S01.035	brachyurin-T
S01.036	granzyme O
S01.037	kallikrein mK5 (Mus sp.)
S01.038	kallikrein mK21 (Mus musculus)

(continued)

CODE	Protease name
S01.039	kallikrein mK22 (Mus musculus)
S01.040	chymotrypsin-like enzyme (Lepidoptera)
S01.041	kallikrein mK11 (Mus musculus)
S01.042	intestinal serine peptidase (rodent)
S01.045	TESP2 peptidase (Mus musculus)
S01.047	adrenal secretory serine peptidase
S01.048	Xesp-1 g.p. (Xenopus laevis)
S01.049	Xesp-2 g.p. (Xenopus laevis)
S01.050	XMT-SP1 g.p. (Xenopus laevis)
S01.052	kallidin-releasing enzyme (Bitis arietans)
S01.054	tryptase delta 1 (Homo sapiens)
S01.055	trypsin 5 (mouse numbering)
S01.057	trypsin 8 (mouse numbering)
S01.058	trypsin 9 (mouse numbering)
S01.059	trypsin 10 (mouse numbering)
S01.060	trypsin 11 (mouse numbering)
S01.061	trypsin 12 (mouse numbering)
S01.062	trypsin 15 (mouse numbering)
S01.063	trypsin 16 (mouse numbering)
S01.064	trypsin 20 (mouse numbering)
S01.065	kallikrein mK2 (Mus musculus)
S01.066	kallikrein mGk4 (Mus musculus)
S01.067	kallikrein mK8 (Mus musculus)
S01.068	kallikrein mK14 (Mus musculus)
S01.069	kallikrein mK24 (Mus musculus)
S01.070	kallikrein mK26 (Mus musculus)
S01.071	kallikrein mK9 (Mus musculus)
S01.072	matriptase-3
S01.073	mouse glandular kallikrein 27
S01.074	marapsin
S01.075	tryptase homologue 2 (Homo sapiens)
S01.076	tryptase homologue 3 (Homo sapiens)
S01.079	transmembrane peptidase, serine 3
S01.081	kallikrein hK15 (Homo sapiens)
S01.082	spermosin (Halocynthia roretzi)
S01.084	mouse kallikrein 10
S01.086	30kP peptidase A (Bombyx-type)
S01.087	mosaic serine peptidase long-form

(continued)

CODE	Protease name
S01.090	hypodermin B
S01.091	natural killer cell peptidase 1 (Rattus norvegicus)
S01.092	trypsin Va (rodent)
S01.093	trypsin Vb (Rattus norvegicus)
S01.094	trypsin 1 (Rattus-type)
S01.095	vascular chymase (Rattus norvegicus)
S01.097	granzyme-like protein 1 (Rattus norvegicus)
S01.099	testis serine peptidase 4
S01.100	tryptase-6 (Mus musculus)
S01.101	trypsin (Streptomyces sp.)
S01.102	trypsin (Streptomyces erythreaus)
S01.103	trypsin (fungal)
S01.104	1700007n14rik protein (Mus musculus)
S01.108	tryptase-5 (Mus musculus)
S01.109	astrovirus serine peptidase
S01.110	trypsin alpha (insect)
S01.111	hypodermin A
S01.112	trypsin (invertebrate)
S01.113	vitellin-degrading endopeptidase (Bombyx-type)
S01.114	trypsin theta (insect)
S01.115	trypsin iota (insect)
S01.116	trypsin zeta (insect)
S01.117	trypsin eta (insect)
S01.118	tryptase (mammalian, non-human)
S01.119	trypsin 2 (anionic) (Rattus norvegicus)
S01.120	trypsin 2 (mammalian, non-human, non-rodent)
S01.121	hypodermin C
S01.122	brachyurin-C
S01.123	euphauserase
S01.124	trypsin (fish)
S01.125	trypsin X (fish)
S01.127	cationic trypsin (Homo sapiens-type)
S01.128	trypsin (Petromyzon-type)
S01.129	trypsin 4 (Mus musculus)
S01.130	trypsin (mosquito type)
S01.131	neutrophil elastase
S01.132	mannan-binding lectin-associated serine peptidase-3
S01.133	cathepsin G

(continued)

CODE	Protease name
S01.134	myeloblastin
S01.135	granzyme A
S01.136	granzyme B, rodent-type
S01.137	granzyme C
S01.139	granzyme M
S01.140	chymase (human-type)
S01.141	mast cell peptidase 1 (rodent)
S01.142	duodenase
S01.143	tryptase alpha
S01.144	cercarial elastase (Schistosoma)
S01.145	mastin
S01.146	granzyme K
S01.147	granzyme H
S01.148	mast cell peptidase 9 (Rattus norvegicus)
S01.149	mast cell peptidase 4 (mouse numbering)
S01.150	mast cell peptidase 5 (mouse numbering)
S01.151	trypsin 1
S01.152	chymotrypsin B
S01.153	pancreatic elastase
S01.154	pancreatic endopeptidase E
S01.155	pancreatic elastase II
S01.156	enteropeptidase
S01.157	chymotrypsin C
S01.159	prostasin
S01.160	kallikrein hK1
S01.161	kallikrein hK2 (Homo sapiens)
S01.162	kallikrein hK3
S01.163	kallikrein mK16 (Mus musculus)
S01.164	mouse kallikrein 1
S01.165	kallikrein rK10 (Rattus norvegicus)
S01.166	chymotrypsin m-type 1 (insect)
S01.167	mouse kallikrein 6
S01.168	chymotrypsin m-type 2 (insect)
S01.170	7S nerve growth factor gamma subunit (Mus sp.)
S01.171	kallikrein 1 (Equus caballus)
S01.172	tonin
S01.173	kallikrein 13 (Mus musculus)
S01.174	mesotrypsin

(continued)

CODE	Protease name
S01.176	batroxobin
S01.177	crotalase
S01.178	Ancrod
S01.179	bothrombin
S01.180	platelet-aggregating venom endopeptidase
S01.181	bilineobin
S01.183	trypsin IV (Rattus norwegicus)
S01.184	factor V activator (Daboia russellii)
S01.186	venom plasminogen activator (Trimeresurus sp.)
S01.187	peptidase 6 (Dermatophagoides sp.)
S01.188	capillary permeability-increasing enzyme-2 (Gloydius-type)
S01.189	complement component C1r-like peptidase
S01.190	tissue kallikrein (Mastomys natalensis)
S01.191	complement factor D
S01.192	complement component activated C1r
S01.193	complement component activated C1s
S01.194	complement component 2
S01.196	complement factor B
S01.198	mannan-binding lectin-associated serine peptidase 1
S01.199	complement factor I
S01.200	Snake endopeptidase (Insecta)
S01.201	Easter endopeptidase
S01.202	Gastrulation-defective g.p. (Drosophila melanogaster)
S01.203	CG3066 protein (Drosophila melanogaster)
S01.204	prophenoloxidase-activating endopeptidase (Holotrichia diomphalia)
S01.205	pancreatic endopeptidase E form B
S01.206	pancreatic elastase II form B (Homo sapiens)
S01.207	9930019B18Rik protein
S01.209	complement component C1rB (Mus musculus)
S01.210	complement component C1sB (Mus musculus)
S01.211	coagulation factor XIIa
S01.212	plasma kallikrein
S01.213	coagulation factor XIa
S01.214	coagulation factor IXa
S01.215	coagulation factor VIIa
S01.216	coagulation factor Xa
S01.217	thrombin
S01.218	protein C (activated)

(continued)

CODE	Protease name
S01.219	coagulation factor C (horseshoe crab), activated
S01.220	coagulation factor B (Limulus, Tachypleus), activated
S01.221	clotting enzyme (Tachypleus)
S01.222	coagulation factor G (Tachypleus), activated
S01.223	acrosin
S01.224	hepsin
S01.225	Stubble endopeptidase (Insecta)
S01.228	hepatocyte growth factor activator
S01.229	mannan-binding lectin-associated serine peptidase 2
S01.231	u-plasminogen activator
S01.232	t-plasminogen activator
S01.233	plasmin
S01.234	peptidase 3 (Dermatophagoides-type)
S01.235	acutobin
S01.236	neurosin
S01.237	neurotrypsin
S01.239	plasminogen activator (Desmodus-type)
S01.240	oviductin
S01.243	lumbrokinase
S01.244	neuropsin
S01.245	ovochymase
S01.246	kallikrein hK10 (Homo sapiens)
S01.247	epitheliasin
S01.248	putative peptidase similar to natural killer cell peptidase 1 (Rattus horwegicus)
S01.250	testis serine peptidase 6
S01.251	prostase
S01.252	brain-specific serine peptidase 4
S01.253	halystase
S01.254	mast cell peptidase 8 (Mus musculus)
S01.255	mekratin
S01.256	chymopasin
S01.257	kallikrein hK11
S01.258	trypsin-2 (Homo sapiens)
S01.260	B1598 endopeptidase
S01.261	streptogrisin A
S01.262	streptogrisin B
S01.263	SAM-P20 peptidase (Streptomyces sp.)
S01.265	streptogrisin C

(continued)

CODE	Protease name
S01.266	streptogrisin D
S01.267	streptogrisin E
S01.268	alpha-lytic endopeptidase
S01.269	glutamyl endopeptidase I
S01.270	exfoliatin A
S01.271	glutamyl endopeptidase BL
S01.272	glutamyl endopeptidase BS
S01.273	peptidase Do
S01.274	DegQ
S01.275	DegS
S01.276	yeast-lytic endopeptidase (Rarobacter)
S01.277	HtrA1 peptidase
S01.278	HtrA2 peptidase
S01.279	DegP2 peptidase (chloroplast)
S01.280	lysyl endopeptidase (bacteria)
S01.281	arginyl endopeptidase
S01.282	SplB g.p. (Staphylococcus aureus)
S01.283	SplC g.p. (Staphylococcus aureus)
S01.284	HtrA3 peptidase
S01.285	HtrA4 peptidase
S01.286	1300019n10rik protein (Mus musculus)
S01.287	kallikrein rK12 (Rattus norvegicus)
S01.288	kallikrein rK8 (Rattus norvegicus)
S01.289	arginine esterase (Canis familiaris)
S01.290	renal kallikrein (Mastomys natalensis)
S01.291	LOC144757 peptidase (Homo sapiens)
S01.292	HAT-like putative peptidase 2
S01.294	HAT-like putative peptidase 3
S01.297	mouse kallikrein 15
S01.298	trypsin C
S01.300	stratum corneum chymotryptic enzyme
S01.302	matriptase
S01.303	mast cell peptidase-11 (rodent)
S01.304	mast cell peptidase-9 (Mus musculus)
S01.305	prophenoloxidase-activating endopeptidase (Bombyx-type)
S01.306	kallikrein hK13
S01.307	kallikrein hK9
S01.308	matriptase-2

(continued)

CODE	Protease name
S01.309	umbelical vein peptidase
S01.311	LCLP peptidase
S01.313	spinesin
S01.314	strypsin-1
S01.315	strypsin-2
S01.316	26 kDa endopeptidase (Sarcophaga peregrina)
S01.317	testis serine peptidase 2 (Mus musculus)
S01.318	marapsin-2
S01.319	complement factor D-like putative peptidase
S01.325	epidermis-specific SP-like putative peptidase
S01.326	testis serine peptidase 5
S01.327	testis serine peptidase 1
S01.328	Try10-like trypsinogen
S01.330	catroxase I
S01.331	pallabin
S01.332	pallabin 2
S01.333	pallase
S01.334	alpha-fibrinogenase (Vipera lebetina)
S01.335	calobin (Gloydius sp.)
S01.336	catroxobin I
S01.337	cerastobin
S01.338	salmobin
S01.339	cerastotin
S01.340	serpentokallikrein-1 (Trimeresurus mucrosquamatus)
S01.341	brevinase
S01.342	cerastocytin
S01.343	mucofibrase 1
S01.344	mucofibrase 4
S01.345	mucofibrase 5
S01.346	okinaxobin I
S01.347	contortrixobin
S01.348	acubin
S01.349	acubin2
S01.350	salmonase
S01.352	elegaxobin (Trimeresurus elegans)
S01.353	KN-BJ endopeptidase 1 (Bothrops jararaca)
S01.354	KN-BJ endopeptidase 2 (Bothrops jararaca)
S01.355	elegaxobin II (Trimeresurus elegans)

(continued)

CODE	Protease name
S01.356	afaacytin (Cerastes cerastes)
S01.357	polyserase-IA protein (unit 1)
S01.358	polyserase-IA protein (unit 2)
S01.360	complement component ClsA (Mus musculus)
S01.361	peptidase similar to prostasin (Mus musculus)
S01.362	testis serine peptidase 2 (Homo sapiens)
S01.363	hypothetical acrosin-like peptidase (Homo sapiens)
S01.364	htrA-like peptidase (Listeria-type)
S01.366	HISP peptidase (Haemaphysalis longicornis)
S01.398	granzyme D (Mus musculus)
S01.399	granzyme E (Mus sp.)
S01.401	granzyme F (Mus musculus)
S01.402	granzyme G (Mus musculus)
S01.404	granzyme RNKP-7 (Rattus)
S01.405	kallikrein rK1 (Rattus)
S01.406	kallikrein rK7 (Rattus)
S01.407	kallikrein rK9 (Rattus)
S01.410	kallikrein K-32 (Rattus norvegicus)
S01.412	CHY1 peptidase (Metarhizium anisopliae)
S01.413	prophenoloxidase-activating endopeptidase (Pacifastacus leniusculus)
S01.417	testis-specific serine peptidase-1
S01.418	kallikrein 5-like peptidase
S01.419	plasma kallikrein-like peptidase
S01.420	prothrombin activator (Lonomia sp.)
S01.421	Persephone endopeptidase (Drosophila melanogaster)
S01.422	fibrinolytic enzyme A (Annelida)
S01.423	SprE glutamyl peptidase (Enterococcus faecalis)
S01.424	serine peptidase SP-28 (Ctenocephalides felis)
S01.425	trocarin D
S01.426	hopsarin D (Hoplocephalus stephensi)
S01.427	prophenoloxidase-activating endopeptidase (Manduca-type)
S01.428	LV-Ka endopeptidase
S01.429	factor V activating enzyme (Vipera lebetina)
S01.430	gabonase (Bitis gabonica)
S01.431	SFase-2 endopeptidase (Streptomyces fradiae)
S01.432	gyroxin (Crotalus durissus terrificus)
S01.433	Bothrops peptidase A (Bothrops jararaca)
S01.434	Nma111 endopeptidase (Saccharomyces cerevisiae)

(continued)

CODE	Protease name
S01.435	gilatoxin (Heloderma horridum)
S01.436	DESC4 peptidase
S01.437	cod chymotrypsin B
S01.438	fire ant chymotrypsin
S01.439	cortical granule serine peptidase 1 (Strongylocentrotus sp.)
S01.440	Vn50 peptidase (Cotesia rubecula)
S01.441	SppA1 peptidase (Arabidopsis thaliana)
S01.442	HtrA stress response protease (Brucella-type)
S01.443	glutamyl endopeptidase BI
S01.444	hemolymph proteinase 14 (Manduca sexta)
S03.001	togavirin
S06.001	IgA1-specific serine endopeptidase (Neisseria sp.)
S06.002	EspP g.p. (Escherichia coli)
S06.003	Tsh peptidase (Escherichia coli)
S06.004	Pet peptidase
S06.005	Pic peptidase (Shigella flexneri)
S06.006	Hap serine peptidase
S06.007	IgA1-specific serine peptidase type 1 (Haemophilus sp.)
S06.008	IgA1-specific serine peptidase type 2 (Haemophilus sp.)
S06.009	EatA peptidase (Escherichia coli)
S06.010	EspC peptidase
S07.001	flavivirin
S08.001	subtilisin Carlsberg
S08.002	mesentericopeptidase
S08.003	subtilisin lentus
S08.004	wprA g.p. (Bacillus-type)
S08.005	peptidase Q (Bacillus pumilis)
S08.006	P69 endopeptidase
S08.007	thermitase
S08.009	subtilisin Ak1
S08.010	M-peptidase (Bacillus sp. KSM-K16)
S08.011	kexin-like peptidase (Pneumocystis carinii)
S08.012	subtilisin-like peptidase 1 (Plasmodium sp.)
S08.013	subtilisin-like peptidase 2 (Plasmodium-type)
S08.014	ALE1 endopeptidase (Arabidopsis thaliana)
S08.016	WF146 peptidase (Bacillus sp. WF146)
S08.017	bacillopeptidase F
S08.018	cell envelope-associated peptidase (Lactobacillus sp.)

(continued)

CODE	Protease name
S08.019	lactocepin I
S08.020	C5a peptidase
S08.021	fervidolysin
S08.022	basic serine peptidase (Dichelobacter)
S08.023	acidic serine peptidase V5 (Dichelobacter)
S08.024	trepolisin
S08.025	antigen Pen ch 13 (Penicillium chrysogenum)
S08.026	nasp g.p. (Dermatophilus congolensis)
S08.030	lspA peptidase
S08.031	blisterase
S08.032	Psp3 protein (Schizosaccharomyces pombe)
S08.034	subtilisin BPN'
S08.035	subtilisin J
S08.036	subtilisin E
S08.037	subtilisin DY
S08.038	alkaline peptidase (Bacillus alcalophilus)
S08.039	proprotein convertase 9
S08.042	subtilisin amylosacchariticus
S08.043	HreP peptidase (Yersinia enterocolitica)
S08.044	subtilisin NAT
S08.045	subtilisin ALP 1
S08.046	subtilisin aprM
S08.047	kpc-1 proprotein convertase
S08.048	furin-1 (insect)
S08.049	Furin-2 g.p. (Drosophila melanogaster)
S08.050	exopeptidase A
S08.051	aqualysin 1
S08.052	cerevisin
S08.053	oryzin
S08.054	endopeptidase K
S08.055	alkaline endopeptidase (Yarrowia lipolytica)
S08.056	cuticle-degrading endopeptidase
S08.057	thermomycolin
S08.058	subtilisin-like peptidase (Ophiostoma sp.)
S08.059	NisP lantibiotic leader peptidase (Lactococcus lactis)
S08.060	EpiP lantibiotic leader peptidase (Staphylococcus epidermidis)
S08.061	peptidase T
S08.062	antigen Pen c 1-type peptidase

(continued)

CODE	Protease name
S08.063	site-1 peptidase
S08.064	PrtA g.p. (Streptococcus pneumoniae)
S08.065	MutP lantibiotic leader peptidase (Streptococcus mutans)
S08.067	Apr g.p. (Alteromonas sp. O-7)
S08.068	SphB1 autotransporter (Bordetella sp.)
S08.069	SAM-P45 peptidase (Streptomyces sp.)
S08.070	kexin
S08.071	furin
S08.072	proprotein convertase 1
S08.073	proprotein convertase 2
S08.074	proprotein convertase 4
S08.075	PACE4 proprotein convertase
S08.076	proprotein convertase 5
S08.077	proprotein convertase 7
S08.078	vitellogenin convertase (Diptera)
S08.079	PrcA peptidase
S08.080	kexin-like peptidase (Tachypleus)
S08.083	CP70 cold-active peptidase (Flavobacterium balustinum)
S08.084	SDD1 peptidase
S08.085	PepP lantibiotic leader peptidase (Staphylococcus epidermidis)
S08.086	CylP/CylA lantibiotic leader peptidase (Enterococcus faecalis)
S08.087	EciP lantibiotic leader peptidase (Staphylococcus epidermidis)
S08.090	tripeptidyl-peptidase II
S08.091	tripeptidyl-peptidase S
S08.092	cucumisin
S08.093	LasP lantibiotic leader peptidase (Lactobacillus sakei)
S08.094	subtilisin extracellular homologue (Serratia)
S08.095	ElkP lantibiotic leader peptidase (Staphylococcus epidermidis)
S08.096	subtilisin homologue (Staphylothermus)
S08.097	peptidase C1 (Glycine max)
S08.098	subtilisin sendai
S08.100	pyrolysin
S08.101	halolysin 1
S08.102	halolysin R4
S08.104	AF70 peptidase (Picea abies)
S08.105	aerolysin
S08.106	stetterlysin
S08.107	peptidase MprA (Burkholderia-type)

(continued)

CODE	Protease name
S08.108	GSP peptidase (Clostridium sp.)
S08.109	C51E3.7B protein (Caenorhabditis elegans)
S08.110	StmPr1 endopeptidase (Stenotrophomonas-type)
S08.111	AprP peptidase (Pseudomonas aeruginosa)
S08.112	ARA12 g.p. (Arabidopsis thaliana)
S08.113	sfericase (Bacillus sphaericus)
S08.114	endopeptidase Vpr (Bacillus-type)
S08.115	subtilisin-like peptidase 3 (Microsporum-type)
S08.116	lactocepin III
S08.117	FT peptidase
S08.118	PrtB peptidase (Lactobacillus delbrueckii subsp. bulgaricus)
S08.119	AIR3 peptidase
S08.120	Aoz1 peptidase (Arthrobotrys oligospora)
S08.121	cytotoxin SubA
S08.122	subtilisin-like peptidase 3 (Plasmodium sp.)
S08.123	KP-43 peptidase (Bacillus sp.)
S09.001	prolyl oligopeptidase
S09.002	prolyl oligopeptidase homologue (Pyrococcus sp.)
S09.003	dipeptidyl-peptidase IV (eukaryote)
S09.004	acylaminoacyl-peptidase
S09.005	dipeptidyl aminopeptidase A
S09.006	dipeptidyl aminopeptidase B (fungus)
S09.007	fibroblast activation protein alpha subunit
S09.008	dipeptidyl peptidase IV (Aspergillus sp.)
S09.010	oligopeptidase B
S09.012	dipeptidyl-peptidase V
S09.013	dipeptidyl-peptidase IV (bacteria)
S09.014	dipeptidyl aminopeptidase B1 (Pseudomonas sp.)
S09.016	S9 homologue (invertebrate)
S09.017	prolyl tripeptidyl peptidase
S09.018	dipeptidyl-peptidase 8
S09.019	dipeptidyl-peptidase 9
S09.021	glutamyl endopeptidase (plant)
S09.051	FLJ1 putative peptidase
S09.056	dipeptidyl-peptidase IV, membrane-type (Giardia intestinalis)
S09.057	apsC g.p. (Aspergillus niger N400)
S09.061	C14orf29 protein
S09.062	hypothetical protein

(continued)

CODE	Protease name
S09.063	hypothetical esterase/lipase/thioesterase (Mus musculus)
S09.065	protein bat5
S09.067	D230019K24Rik protein (Mus musculus)
S10.001	carboxypeptidase Y
S10.002	serine carboxypeptidase A
S10.003	vitellogenic carboxypeptidase-like protein
S10.004	serine carboxypeptidase C
S10.005	serine carboxypeptidase D
S10.007	kex carboxypeptidase
S10.008	carboxypeptidase S1 (Penicillium janthinellum)
S10.009	carboxypeptidase III (plant)
S10.010	serine carboxypeptidase Z (Absidia zachae)
S10.011	serine carboxypeptidase P
S10.012	Sxa2 carboxypeptidase
S10.013	RISC peptidase
S11.001	D-Ala-D-Ala carboxypeptidase A
S11.002	murein-DD-endopeptidase
S11.003	penicillin-binding protein 6
S11.004	K15 DD-transpeptidase (Streptomyces sp.)
S11.005	D-Ala-D-Ala carboxypeptidase DacF
S11.006	D,D-carboxypeptidase PBP3 (Streptococcus sp.)
S12.001	D-Ala-D-Ala carboxypeptidase B
S12.002	aminopeptidase DmpB
S12.003	alkaline D-peptidase (Bacillus sp.)
S12.004	LACT-1 peptidase
S13.001	D-Ala-D-Ala peptidase C
S13.002	D-Ala-D-Ala carboxypeptidase (Actinomadura-type)
S13.003	D-Ala-D-Ala carboxypeptidase PBP3 (Neisseria sp.)
S14.001	endopeptidase Clp (type 1)
S14.002	endopeptidase Clp (type 2)
S14.003	endopeptidase Clp (type 3)
S14.004	endopeptidase Clp (type 4)
S14.005	endopeptidase Clp (type 5)
S14.006	endopeptidase Clp (type 6)
S14.007	endopeptidase Clp (type 7)
S14.008	ClpP1 endopeptidase (Streptomyces-type)
S14.009	ClpP2 endopeptidase (Streptomyces-type)
S15.001	Xaa-Pro dipeptidyl-peptidase

(continued)

CODE	Protease name
S16.001	Lon-A peptidase
S16.002	PIM1 endopeptidase
S16.003	endopeptidase La homologue (type 3)
S16.004	Lon peptidase (type 4)
S16.005	Lon-B peptidase
S21.001	assemblin
S21.002	cytomegalovirus assemblin
S21.003	Epstein-Barr virus-type assemblin
S21.004	herpesvirus 6-type assemblin
S21.005	Varicella zoster assemblin
S21.006	herpesvirus 8-type assemblin
S24.001	repressor LexA
S24.002	phage lambda repressor protein
S24.003	UmuD protein
S24.004	RvuZ homologue protein (Rattus)
S26.001	signal peptidase I
S26.002	mitochondrial inner membrane peptidase 1
S26.003	signal peptidase SipS
S26.004	signal peptidase SipT
S26.005	signal peptidase SipU
S26.006	signal peptidase SipV
S26.007	signal peptidase SipP
S26.008	thylakoidal processing peptidase
S26.009	signalase (eukaryote) 18 kDa component
S26.010	signalase (eukaryote) 21 kDa component
S26.011	signal peptidase SipW (Bacillus-type)
S26.012	mitochondrial inner membrane peptidase 2
S26.013	mitochondrial signal peptidase (metazoa)
S26.014	TraF peptidase
S26.015	Streptococcus-type signal peptidase
S26.016	signal peptidase SpsB (Staphylococcus aureus)
S26.017	archaean signal peptidase (Methanococcus voltae)
S26.018	signal peptidase SipM (Bacillus megaterium)
S26.019	similar to type-I signal peptidase
S28.001	lysosomal Pro-Xaa carboxypeptidase
S28.002	dipeptidyl-peptidase II
S28.003	thymus-specific serine peptidase
S29.001	hepacivirin

(continued)

CODE	Protease name
S29.002	hepatitis G virus NS3 endopeptidase
S30.001	potyvirus P1 peptidase
S31.001	pestivirus NS3 polyprotein peptidase
S32.001	equine arteritis virus serine endopeptidase
S33.001	prolyl aminopeptidase
S33.002	tripeptidyl-peptidase A (Streptomyces sp.)
S33.003	leucine aminopeptidase pepL
S33.004	prolinase (Lactobacillus sp.)
S33.005	tricorn interacting factor F1
S33.006	tripeptidyl-peptidase B
S33.007	tripeptidyl-peptidase C (Streptomyces sp.)
S33.008	prolyl aminopeptidase 2
S33.010	SCO7095 endopeptidase (Streptomyces coelicolor A3(2))
S33.011	epoxide hydrolase-like putative peptidase
S33.012	Loc328574-like protein
S37.001	PS-10 peptidase
S39.001	sobemovirus peptidase
S39.002	luteovirus peptidase
S41.001	C-terminal processing peptidase-1
S41.002	C-terminal processing peptidase-2
S41.004	C-terminal processing peptidase-3
S41.005	tricorn core peptidase (archaea)
S41.006	tricorn core peptidase (bacteria)
S41.007	ctpB peptidase (Bacillus subtilis)
S45.001	penicillin G acylase precursor
S45.002	cephalosporin acylase precursor
S46.001	dipeptidyl-peptidase 7
S48.001	HetR endopeptidase
S49.001	signal peptide peptidase A
S49.002	sohB endopeptidase
S49.003	protein C (bacteriophage lambda)
S49.004	peptidase IV (Arabidopsis thaliana)
S49.005	protein 1510-N (Pyrococcus horikoshii)
S50.001	infectious pancreatic necrosis birnavirus Vp4 peptidase
S50.002	avian infectious bursal disease birnavirus Vp4 endopeptidase
S50.003	Drosophila X virus Vp4 peptidase
S50.004	blotched snakehead birnavirus Vp4 peptidase
S51.001	dipeptidase E

(continued)

CODE	Protease name
S51.002	alpha-aspartyl dipeptidase (eukaryote)
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S53.006	physarolisin
S53.007	aorsin
S53.008	physarolisin II
S53.009	kumamolisin-As
S54.001	Rhomboid-1 (Diptera)
S54.002	rhomboid-like protein 2
S54.004	aarA protein (Providencia stuartii)
S54.005	rhomboid-like protein 1
S54.006	ventrhoid transmembrane protein
S54.007	Pcp1 protein (Saccharomyces cereviseae)
S54.008	rhomboid-like protein 5
S54.009	PARL peptidase
S54.010	Rhomboid-2 (Drosophila-type)
S54.011	Rhomboid-3 (Drosophila melanogaster)
S54.012	Rhomboid-4 (Drosophila melanogaster)
S54.013	ROM-1 peptidase (Caenorhabditis elegans)
S54.014	rhomboid YqgP (Bacillus subtilis)
S55.001	SpoIVB peptidase
S58.001	aminopeptidase DmpA
S59.001	nucleoporin 145
S60.001	lactoferrin
S62.001	influenza A PA endopeptidase
S63.001	EGF-like module containing mucin-like hormone receptor-like 2
S63.002	CD97 antigen
S63.003	EGF-like module containing mucin-like hormone receptor-like 3
S63.004	EGF-like module containing mucin-like hormone receptor-like 1 (Homo sapiens)
S63.005	FLJ00015 protein (Homo sapiens)
S63.006	FLJ00046 protein (Homo sapiens)
S63.008	EGF-like module containing mucin-like hormone receptor-like 4
S64.001	Ssy5 endopeptidase (Sacchaomyces cerevisiae)
S9C.001	glycylprolyl peptidase (Bacteroides gingivalis)

(continued)

CODE	Protease name
S9F.001	peptidyl-glycinamidase
S9G.002	dog pancreatic collagenase
S9G.005	elastase-like enzyme, platelet
S9G.006	tissue elastase
S9G.009	macrophage chymotrypsin-like endopeptidase
S9G.012	tryase
S9G.013	guanidinobenzoatase
S9G.014	clipsin
S9G.016	thymus chromatin endopeptidase
S9G.018	nuclear histone endopeptidase
S9G.023	ingobsin
S9G.025	snake venom coagulation factor X activator, serine-type (Bungarus fasoatus. Cerastes vipera, Ophiophagus hannah)
S9G.027	scutelarin (Oxyuranus scutellatus)
S9G.031	leucyl endopeptidase (Spinacia oleracea)
S9G.034	metridin
S9G.035	serine endopeptidase (Alternaria)
S9G.036	collagenolytic endopeptidase (Entomophthora)
S9G.038	endopeptidase So
S9G.040	serine endopeptidase (Pseudomonas)
S9G.041	peptidase V (Escherichia coli)
S9G.042	peptidase Mi (Escherichia coli)
S9G.043	peptidase Fa (Escherichia coli)
S9G.049	extracellular serine endopeptidase (Arthrobacter)
S9G.050	peptidase VI (Escherichia coli)
S9G.054	profilaggrin endopeptidase 1
S9G.055	thermostable serine endopeptidase (Sulfolobus)
S9G.056	sporangin
S9G.058	beta-secretase Matsumoto
S9G.060	amelopeptidase
S9G.061	peptidase gp76 (Plasmodium falciparum)
S9G.062	thrombocytin
S9G.063	peptidase In (Escherichia coli)
S9G.064	archealysin
S9G.065	fish muscle prokallikrein
S9G.066	mole salivary kallikrein
S9G.069	apoptotic serine peptidase AP24
S9G.072	erythrocyte membrane high molecular mass peptidase

(continued)

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S9G.075	LasD g.p. (Pseudononas aeruginosa)
S9G.077	serine endopeptidase (Perkinsus marinus)
S9G.079	plan Asp/Glu serine endopeptidase
S9G.081	SP220K peptidase
S9G.082	tryptase Clara
S9G.083	soluble dipeptidyl-peptidase IV
S9G.084	dipeptidyl-peptidase IV beta
S9G.087	jerdonobin (Trimeresurus jerdonii)
S9G.088	jerdofibrase (Trimeresurus jerdonii)
S9G.089	flavovilase (Trimeresurus flavoviridis)
S9G.092	M003 endopeptidase (Bothrops moojeni)
S9G.093	MSP 1 endopeptidase (Bothrops moojeni)
S9G.094	MSP 2 endopeptidase (Bothrops moojeni)
S9G.099	pseutarin C (Pseudonaja textilis)
S9G.100	okinaxobin II (Trimeresurus okinavensis)
S9G.101	habutobin
S9G.103	PofibS endopeptidase (Philodryas olfersii)
T01.002	archaean proteasome, beta component
T01.005	bacterial proteasome, beta component
T01.006	HslV component of HslUV peptidase
T01.007	CodW component of CodWX peptidase
T01.010	proteasome catalytic subunit 1
T01.011	proteasome catalytic subunit 2
T01.012	proteasome catalytic subunit 3
T01.013	proteasome catalytic subunit 1i
T01.014	proteasome catalytic subunit 2i
T01.015	proteasome catalytic subunit 3i
T01.016	RIKEN cDNA 5830406J20
T01.017	protein serine kinase c17 (Homo sapiens)
T02.001	glycosylasparaginase precursor
T02.002	asparaginase
T02.004	taspase-1
T02.005	asparaginase-like sperm autoantigen homolog
T02.006	hypothetical protein flj22316
T03.001	gamma-glutamyltransferase 1 (bacterial)
T03.002	gamma-glutamyltransferase 5 (mammalian)
T03.005	gamma-glutamyltransferase (Drosophila melanogaster)
T03.006	gamma-glutamyltransferase 1 (mammalian)

(continued)

CODE	Protease name
T03.007	gamma-glutamyltransferase CG4829 (Drosophila melanogaster)
T03.008	gamma-glutamyltransferase (plant)
T03.009	gamma-glutamyltransferase (nematode)
T03.010	gamma-glutamyltransferase CG1492 (Drosophila melanogaster)
T03.011	gamma-glutamyltransferase (Schizosaccharomyces)
T03.012	gamma-glutamyltransferase (Saccharomyces)
T03.013	gamma-glutamyltransferase (Synechocystis-type)
T03.014	gamma-glutamyltransferase 2 (bacterial)
T03.015	gamma-glutamyltransferase 2 (Homo sapiens)
T03.016	gamma-glutamyltransferase-like protein 4
T03.017	gamma-glutamyltransferase-like protein 3
T03.018	similar to gamma-glutamyltransferase 1 precursor (Homo sapiens)
T03.020	gamma-glutamyltransferase-like protein A4
T03.022	9030405D14Rik protein (Mus musculus)
T05.001	ornithine acetyltransferase precursor

SEQUENCE LISTING

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Gln Ala Lys Cys Glu Ala Ser Tyr Pro Gly Lys Ile Thr Ser Asn Met
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```

Claims

1. A method for generating a protease with reduced sensitivity towards activity-modulating substances present within an application matrix, comprising

   (a) providing a library of one or more proteases derived from one or more parent proteases,
   (b) contacting the proteases with at least one activity-modulating substance, and
   (c) selecting one or more protease variants with reduced sensitivity towards activity-modulating substances as compared to the parent protease(s).

2. The method of claim 1, wherein in step (b) the protease is contacted either simultaneously or consecutively with the activity-modulating substance and at least one substrate, and the turnover of the substrate by the protease under the influence of the activity-modulating substance is determined.

3. The method of claim 1 or 2, wherein in step (c) the protease variants with reduced sensitivity towards activity-modulating substances are selected by determination of improved IC50 as compared to the parent protease(s).

4. The method according to any of claims 1 to 3, wherein the selection in step (c) is achieved by

   (i) screening for the residual activity of the protease simultaneous to the contact of the proteases with the activity-modulating substance,
   (ii) screening for residual activity of the protease consecutive to the contact of the proteases with the activity-modulating substances, or
   (iii) a combination thereof.

5. The method according to any of claims 1 to 4, which comprises providing the protease library by surface display expression method, preferably by phage display, and

   (i) contacting the displayed proteases with the activity-modulating substances, depleting proteases with sensitivity towards activity modulating substances by immobilisation of complexes comprising the activity-modulating substance and the displayed protease, and selecting for proteases with reduced sensitivity towards activity-modulating substances from the non-immobilized displayed proteases; or
   (ii) contacting the displayed proteases with one or more substrates, enriching of proteases with reduced sensitivity towards activity-modulating substances by immobilisation of complexes comprising the substrate and the displayed protease, and selecting for proteases with reduced sensitivity towards activity-modulating substances from the immobilized displayed proteases.

6. The method according to any of claims 1 to 5, which further comprises (d) selecting for protease variants in step (c) with the additional selection criteria of substantially similar or altered specificity with regard to the substrate as compared to the parent protease(s), or selecting for protease variants in step (c) and modifying the protease variants obtained in step (c) to exhibit a sustatially similar or altered specificity with regard to the substrate as compared to the parent protease and/or the protease variants obtained in step (c).

7. The method according to any of claims 1 to 6, wherein steps (a) to (c) or steps (a) to (d) are repeated cyclically until one or more protease variants with reduced sensitivity towards activity modulating substances are identified, the variants selected in one cycle are used as parent proteases to provide a library for the following cycle, the concentration of the activity-modulating substance is increased from one cycle to the next, and at least two cycles and less than 100 cycles are performed.

8. The method of claim 6, wherein the modifying step comprises rational design, mutagenesis methods such as random mutagenesis, conversion into a protease scaffold that provides the general catalytic activity with variable specificity determining regions (SDRs), chemical or genetic fusion to a binding molecule, or any combination thereof.

9. The method according to any of claims 1 to 8, wherein in step (b) the proteases are contacted with the activity-modulating substances for 1 s to 72 h, preferably for 30 min to 4 h, before the selection of one or more proteases.

10. The method according to any of claims 1 to 9, wherein step (a) comprises providing a library of one or more polynucleotide molecules encoding the proteases, preferably derived form one or more template polynucleotides,

by site-specific or random insertion, deletion or substitution of single or multiple mono-nucleotides, polynucleotides, or nucleotide triplets; homologous in vitro recombination; homologous in-vivo recombination; non-homologous recombination; or a combination thereof; and wherein mutagenesis of the polynucleotide templates is performed at or near the active sites of the protease, or at or near the surface of the protease, or random over the entire polynucleotide molecule; or any combination thereof.

11. The method according to any of claims 1 to 10, wherein

(i) the library of proteases is expressed in a mammalian or non-mammalian cell-line, viral expression system, yeast, fungi, bacteria, preferably Escherichia coli or Bacillus subtilis, or by use of a cell-free expression system, and wherein genotype and phenotype of the proteases of the population are coupled by use of sample carriers that enable compartmentation of samples, and distributing of genotypes into the sample carriers in a multiplicity per compartment that allows sufficient differentiation of phenotypes; and/or
(ii) the at least one activity-modulating substance contacted with the protease library is provided as isolated component, as part of a fraction of the application matrix or as part of the application matrix, or in any combination thereof; and/or
(iii) the application matrix is derived from a human or animal body fluid selected from the group consisting of blood, digestive fluids, preferably intestinal and gastric juice, mucosa, synovial fluid, interstitial fluid, mucosal fluid, cerebrospinal fluid, peritoneal fluid, or from the extracellular matrix; and/or
(iv) the activity-modulating substance is selected from the table 1, and/or is a human protease inhibitor, preferably a serpin selected from the group consisting of alpha1-antitrypsin, alpha1-antichymotrypsin, kallistatin, protein C-inhibitor, leucocyte elastase inhibitor, plasminogen activator inhibitor, maspin, serpin B6, megsin, serpin B9, serpin B10, serpin B11, serpin B12, serpin B13, antithrombin, heparin cofactor, plasminogen activator inhibitor, alpha-2-plasmin inhibitor, C1-inhibitor, neuroserpin, serpin I2 and thyroxin-binding globulin; a cystein protease inhibitors selected from the group consisting of cystatin A, cystatin B, cystatin C, cystatin D, cystatin E/M, cystatin F, cystatin S, cystatin SA, cystatin SN, cystatin G, kininogen inhibitor unit 2 and kininogen inhibitor unit 3; a metallo protease inhibitor selected from the group consisting of TIMP-1, TIMP-2, TIMP-3 and TIMP-4; macroglobulins, preferably alpha2-macroglobulin; BIRC-1; BIRC-2; BIRC-3; BIRC-4; BIRC-5; BIRC-6; BIRC-7 or BIRC-8.

12. The method according to any of claims 1 to 11, wherein the one or more parent proteases

(i) are derived from proteases with a non-naturally occurring catalytic activity of defined specificity engineered by rational design, mutagenesis methods such as random mutagenesis, combination of a protease scaffold that provides the general catalytic activity with variable specificity determining regions (SDRs), chemical or genetic fusion to a binding molecule, or any combination thereof; and/or
(ii) are derived from a protease selected from the group consisting of aspartic, cysteine, serine, metallo and threonine proteases, preferably the protease is derived from a serine protease of the structural class S1, S8, S11, S21, S26, S33 or S51, more preferably from class S1 or S8, a cysteine protease of the structure class C1, C2, C4, C10, C14, C19, C47, C48 or C56, more preferably from class C14, an aspartic protease of the structural class A1, A2 or A26, more preferably from class A1, a metalloprotease of the strucutral class M4 or M10, or the protease is selected from the table 2.

13. The method according to claim 12, wherein the protease is a serine protease of the structural class S1, preferably is a trypsin-like protease, more preferably is derived from a human trypsin, most preferably is derived from the protease according to SEQ ID NO: 5.

14. The method according to any of claims 1 to 13, wherein the parent protease or the protease variant selected in step (c) or (d) is fused to

(i) at least one further proteinacious component, preferably said proteinacious component being selected from the group consisting of binding domains, receptors, antibodies, regulation domains, pro-sequences, and fragments thereof, and/or
(ii) at least one further functional component, preferably said further functional component being selected from the group consisting of polyethylenglycols, carbohydrates, lipids, fatty acids, nucleic acids, metals, metal chelates, and fragments or derivatives thereof.

15. A protease with reduced sensitivity towards activity-modulating substances obtainable by the method according to

any of claims 1 to 14.

16. The protease of claim 15, which

(i) is derived from a serine protease as defined in claim 12 or 13, preferably is derived from human trypsin having the the amino acid sequence shown in SEQ ID N0:5 or a modified form thereof, and/or
(ii) has one or more mutations at positions that correspond structurally or by amino acid sequence homology to the regions 18-28, 33-41, 48-68 and 129-137 in human trypsin, preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 20-26, 35-29, 50-59, 62-66 and 131-135 in human trypsin, and more preferably at one or more of the following positions 22, 23, 24, 37, 52, 57, 64 and 133 numbered according to the amino acid sequence shown in SEQ ID NO:5.

17. The protease of claim 15 or 16, which has at least one mutation selected from Y22T, H23T, F24I or F24V, S37T, E52V, V57A, F64I and D133G, preferably at least 5 mutations selected from S37T, E52V, V57A, F64I and D133G, according to the amino acid sequence shown in SEQ ID NO:5, or mutations at the respective positions which are homologous to said mutations, more preferably has at least three of said mutations, and most preferably is a trypsin mutant of SEQ ID NO:5 having one of the following combination of mutations:

S37T/E52V/V57A/ F64I/D133G,
Y22T/H23T/F24I/S37T/E52V/V57A/F64I/D133G and
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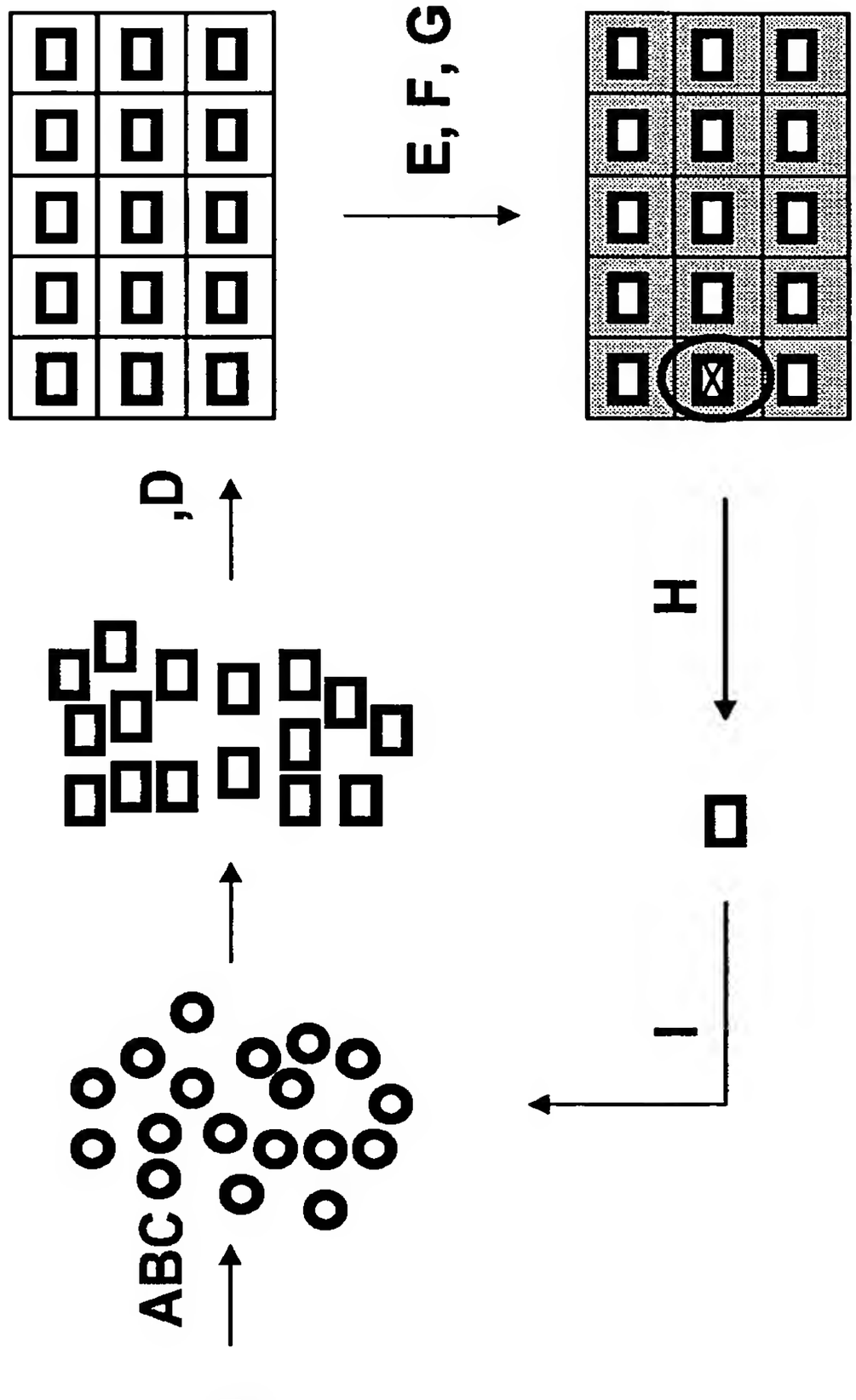
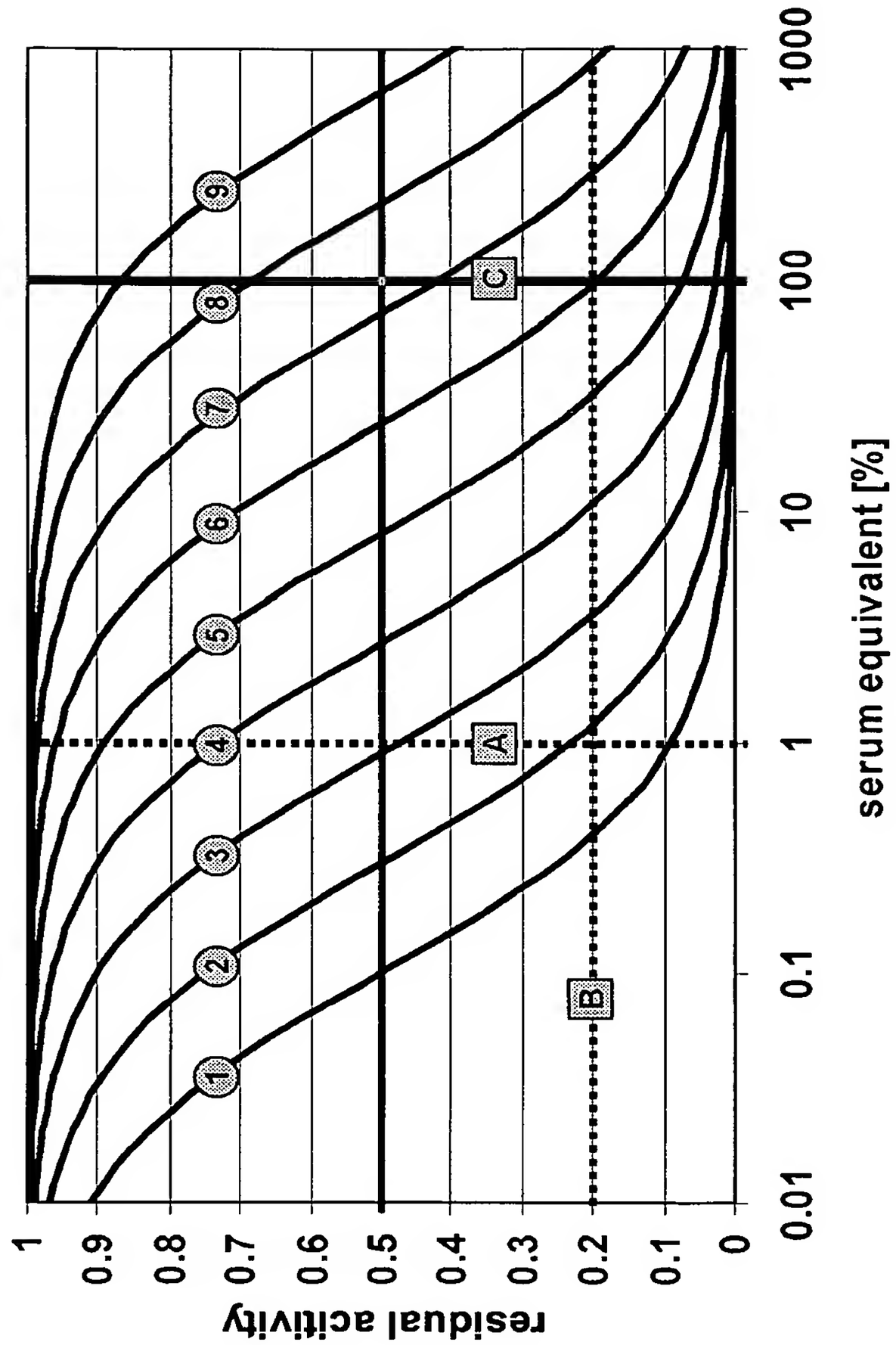


Fig.1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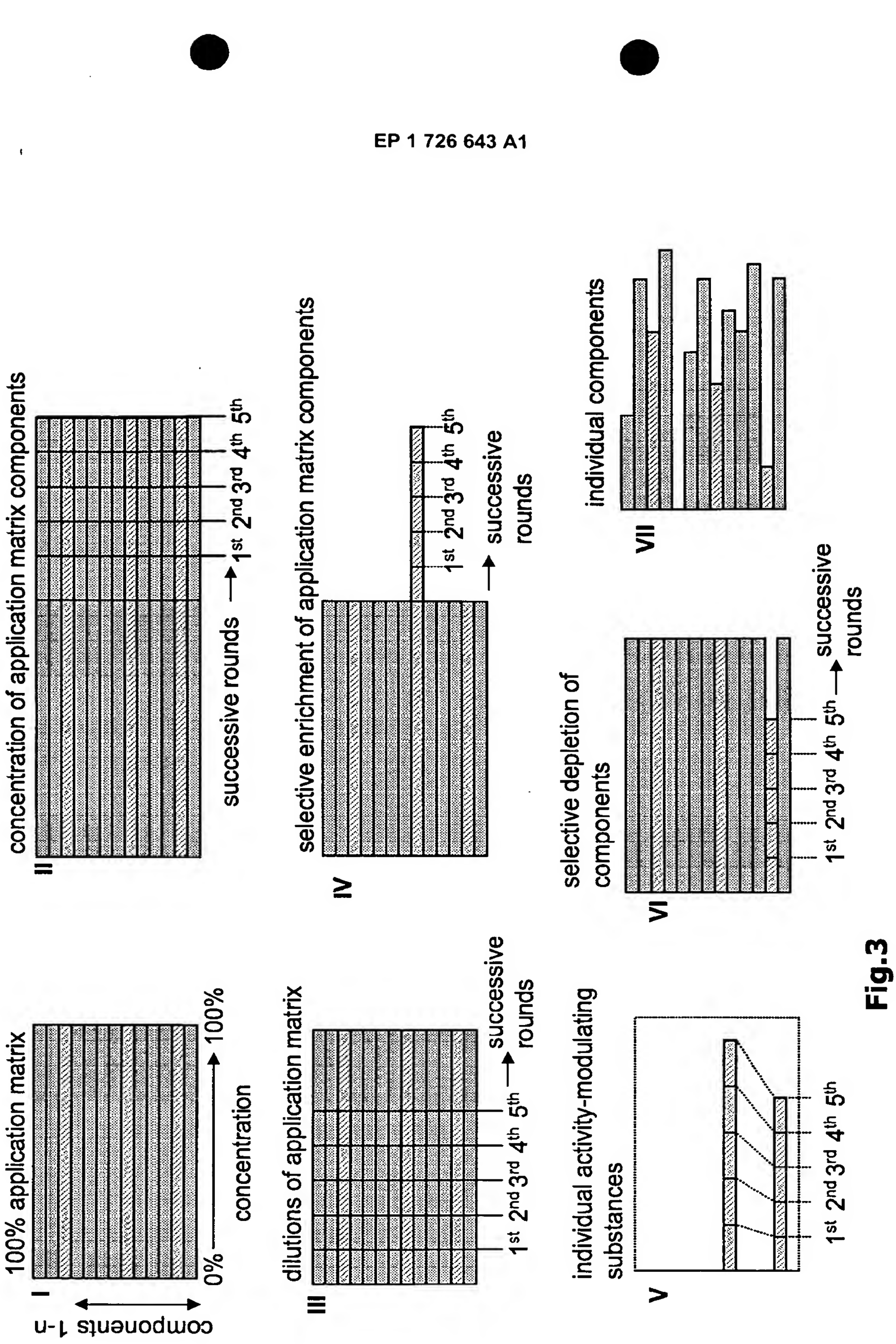
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dilutions of application matrix
1st 2nd 3rd 4th 5th
successive rounds
III
selective enrichment of application matrix components
1st 2nd 3rd 4th 5th
successive rounds
IV
individual activity-modulating substances
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successive rounds
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successive rounds
VI
individual components
VII

Fig.3

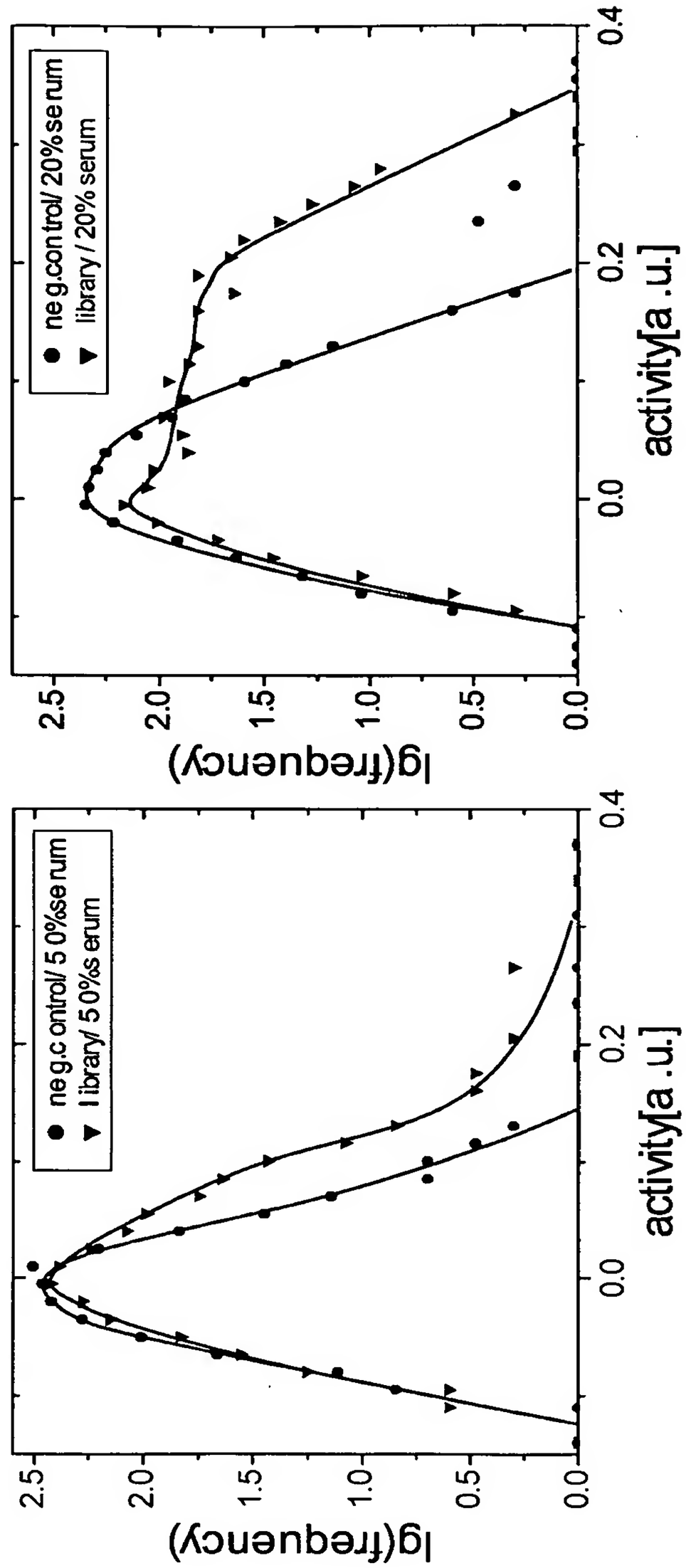
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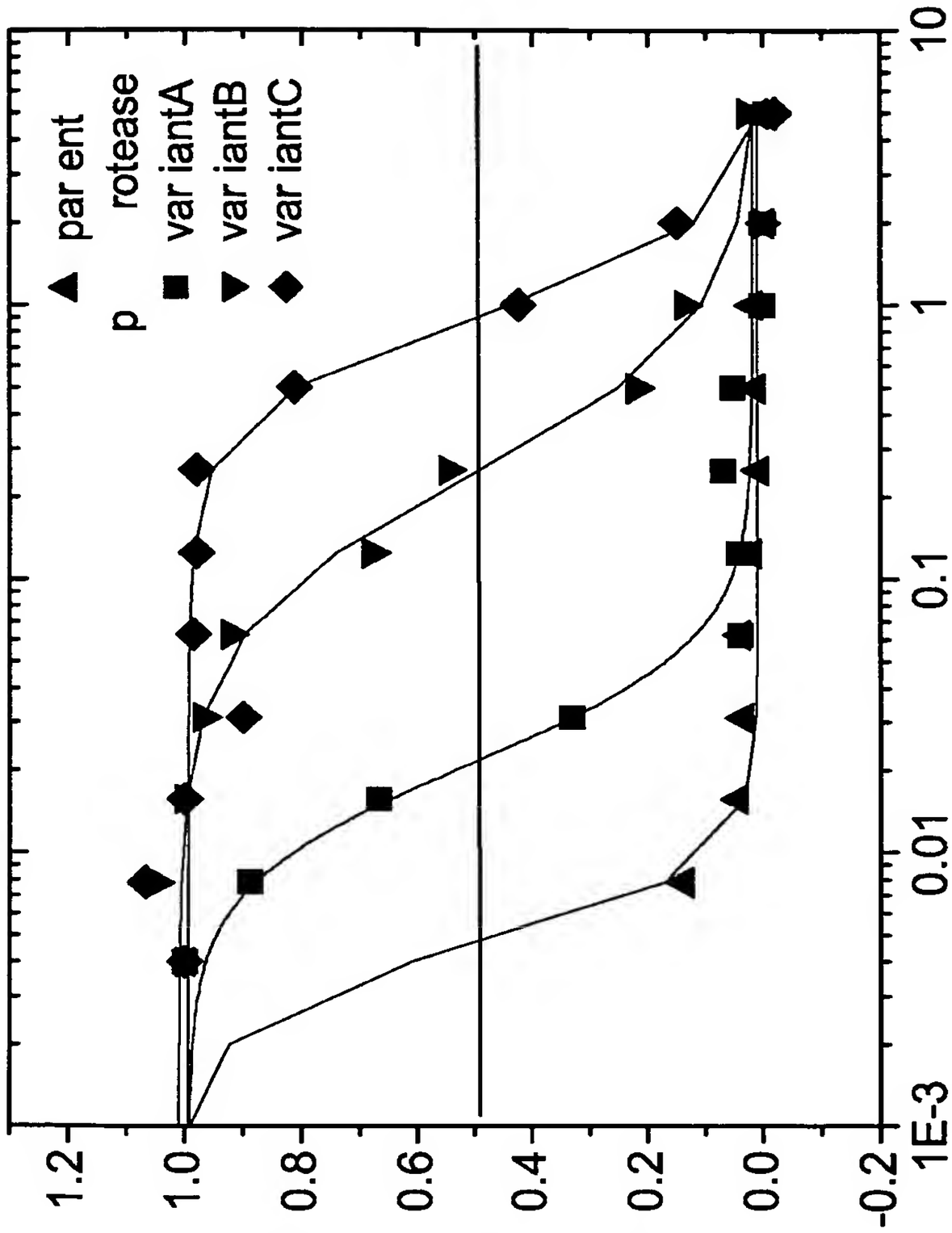
Fig.4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-0.2
E-3
0.01
0.1
1
10
equivalentof serum [%]

Fig.5

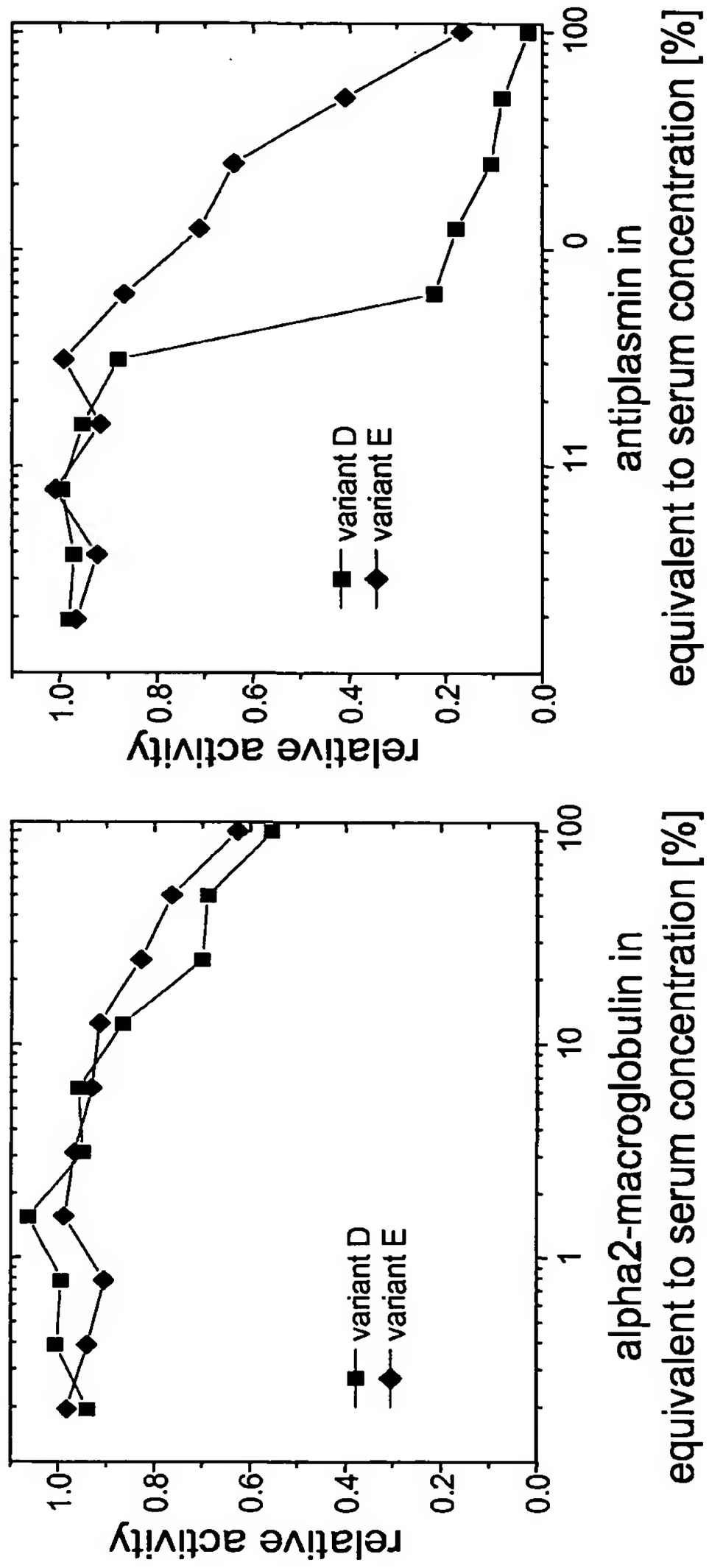
relative activity
1.0
0.8
0.6
0.4
0.2
0.0
variant D
variant E
1
10
100
alpha2-macroglobulin in
equivalent to serum concentration [%]
relative activity
1.0
0.8
0.6
0.4
0.2
0.0
variant D
variant E
1
11
100
antiplasmin in
equivalent to serum concentration [%]

Fig.6

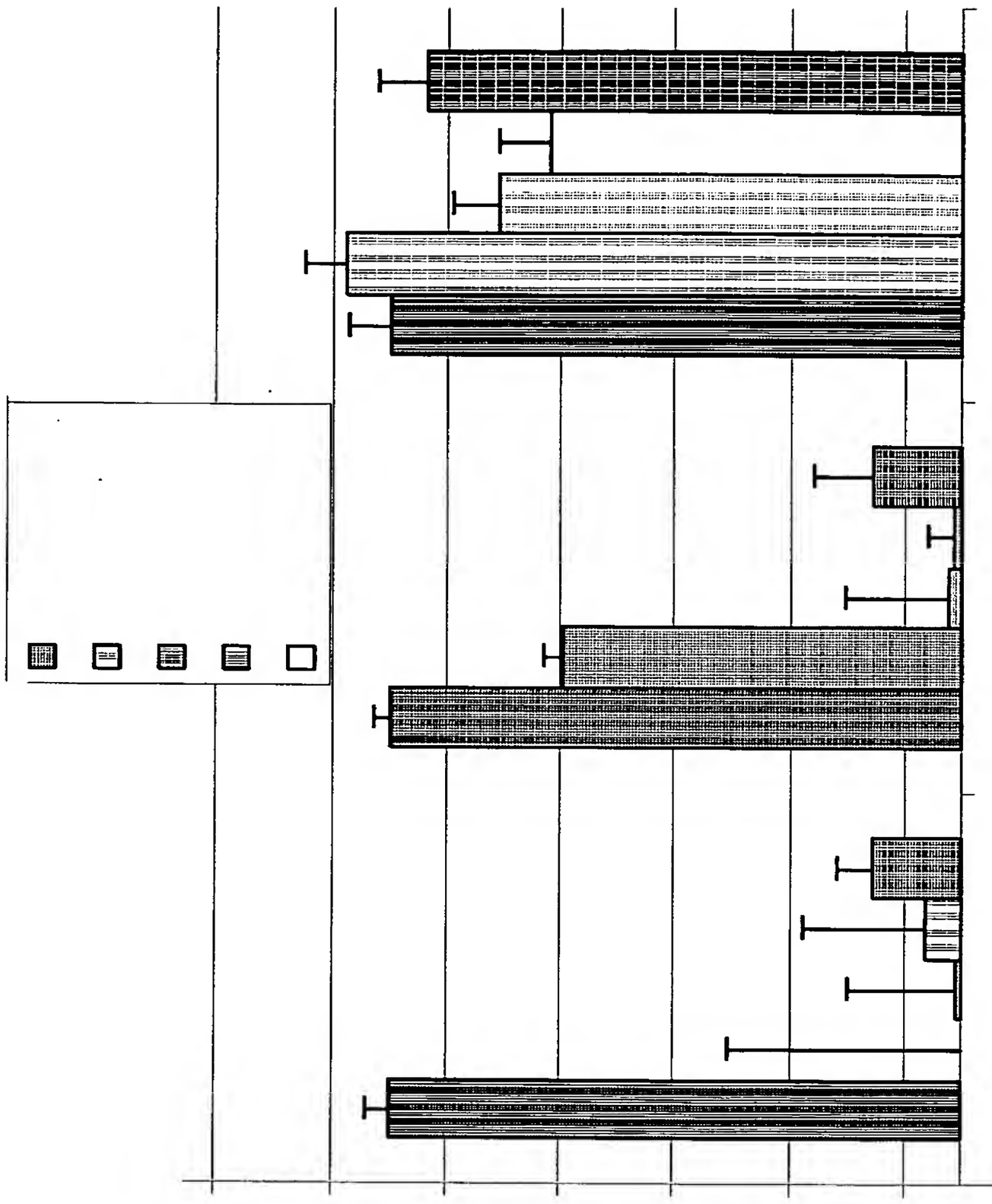

Fig.7

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 05 10 4543

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	WO 02/100337 A (EMORY UNIVERSITY) 19 December 2002 (2002-12-19) See example 3; Table 1	1-4,6-14	C12N9/00 C12N15/00 C12Q1/37
L	JOSSO F ET AL: "A new variant of human prothrombin: Prothrombin Metz, demonstration in a family showing double heterozygosity for congenital hypoprothrombinemia and dysprothrombinemia" HAEMOSTASIS 1982 SWITZERLAND, vol. 12, no. 4, 1982, pages 309-316, XP009052805 Whole document L: Unity		
L	SZMOLA RICHÁRD ET AL: "Human mesotrypsin is a unique digestive protease specialized for the degradation of trypsin inhibitors." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 5 DEC 2003, vol. 278, no. 49, 5 December 2003 (2003-12-05), pages 48580-48589, XP002342456 ISSN: 0021-9258 Whole document L: Unity		TECHNICAL FIELDS SEARCHED (IPC) C12N C12Q
X	WO 02/099387 A (VIROLOGIC, INC) 12 December 2002 (2002-12-12) * the whole document *	1-4,9-12	
X	WO 2004/113521 A (DIREVO BIOTECH AG; HAUPTS, ULRICH; KOLTERMANN, ANDRE; SCHEIDIG, ANDREA) 29 December 2004 (2004-12-29) * the whole document *	1-4,6-14	
	-/--		

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
Munich	26 August 2005	Roscoe, R

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

1 EPO FORM 1503 03.82 (P04C01)

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EUROPEAN SEARCH REPORT

Application Number
EP 05 10 4543

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	US 5 602 021 A (DAVIS ET AL) 11 February 1997 (1997-02-11) Primarily col.11 -----	1-4,6-14	
X	US 6 063 562 A (MELNICK ET AL) 16 May 2000 (2000-05-16) * the whole document * -----	1-4,9-13	
			TECHNICAL FIELDS SEARCHED (IPC)

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
Munich	26 August 2005	Roscoe, R

CATEGORY OF CITED DOCUMENTS

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& : member of the same patent family, corresponding document

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European Patent Office

Application Number
EP 05 10 4543

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1 (part), 2-4, 6-14 (part)

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 05 10 4543

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1(part), 2-4, 6-14(part)

Method for generating a protease with reduced sensitivity towards activity-modulating substances which involves mixing protease, inhibitor and substrate and selecting protease variants (e.g. from wells) which display significant substrate turnover in the presence of the inhibitor.

---

2. claims: 1(part), 5, 6-14(part)

Method for generating a protease with reduced sensitivity towards activity-modulating substances which involves depletion of members of (e.g. phage-display) libraries which display protease by removal of proteases which bind to immobilized modulator and optionally subsequent positive selection for phage which bind to substrate.

---

3. claims: 1(part), 5, 6-14(part)

Method for generating a protease with reduced sensitivity towards activity-modulating substances which involves selection of members of (e.g. phage display) library by binding of the displayed protease to immobilized substrate in the presence of the activity-modulating substance.

---

4. claim: 15(part)

Aspartic Protease which has reduced sensitivity towards activity-modulating substances.
Note: Present division of proteases into invention groups is based on list of protease types in claim 12(ii) which provides the first reference to specific types of proteases. It is noted that each of these groups is in itself large as can be sen from the extensive list of proteases in Table 2 and is hence in itself likely to lack unity. Consequently, should applicant pay additional search fees for any of the invention groups relating to proteases per se, it is virtually inevitable that further invention (sub-)groups will then be identified and further fees requested therefore.

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5. claim: 15(part)

Cysteine Protease which has reduced sensitivity towards activity-modulating substances.

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 05 10 4543

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

6. claims: 15(part), 16, 17

Serine Protease (e.g. Trypsin) which has reduced sensitivity towards activity-modulating substances.

---

7. claim: 15(part)

Metalloprotease which has reduced sensitivity towards activity-modulating substances.

---

8. claim: 15(part)

Threonine Protease which has reduced sensitivity towards activity-modulating substances.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 05 10 4543

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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26-08-2005

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